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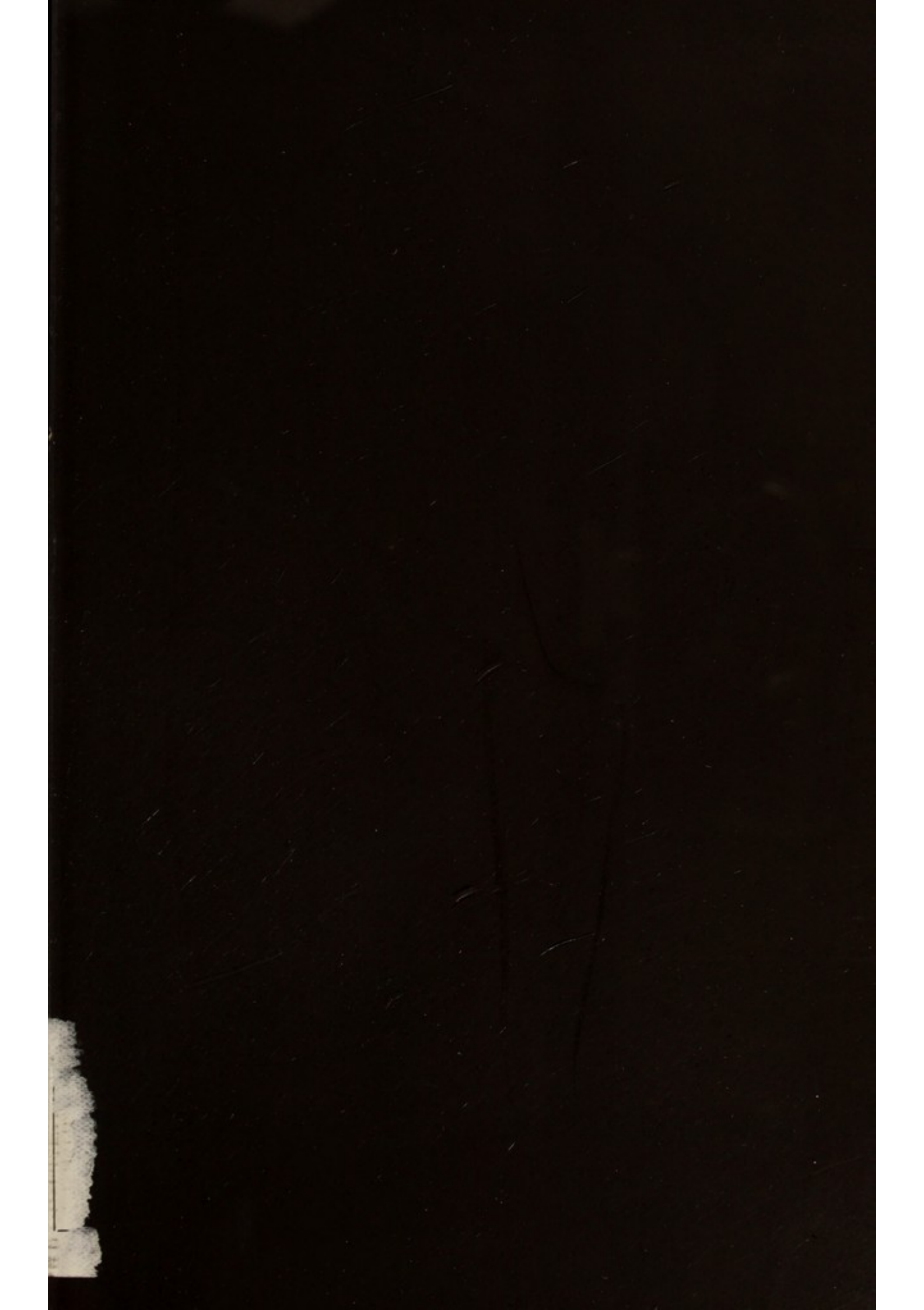
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FERMENTS

AND

THEIR ACTIONS.

BY

CARL OPPENHEIMER, M.D., Ph.D.,

ASSISTANT IN THE PHYSIOLOGICAL INSTITUTE AT ERLANGEN.

TRANSLATED FROM THE GERMAN

By C. AINSWORTH MITCHELL, B.A.(Oxon.), F.I.C.



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P R E F A C E.

THE object of this work is twofold. In the first place, I have made an attempt to formulate a *comprehensive conception of the notion "ferment" on a dynamic basis*, and starting from this standpoint to regard the "*Theory of Ferments*" as a theoretically closed field of discussion. In this conception I have treated the enzymes and organised ferments as a connected whole.

In the second place, I have endeavoured to deal as completely as possible with the material to be found in publications on this subject.

But here a great difficulty presented itself, particularly in the case of processes which are intimately connected with the living cells. For in these processes it is hardly possible to refer with anything approaching completeness to those researches which are of importance to the present subject of *fermentations*, owing to the fact that they are mixed with a great number of investigations on kindred subjects, which are dealt with from a purely *biological* or from a *technical* standpoint. Hence, in discussing the "organised ferments," I have only been able to make a selection from these researches, so as to pick out those results which have a bearing on *fermentation* as such. On the other hand, I have attempted to deal with the literature relating to the "*unorganised ferments*" as fully as possible. To this rule I have made only one exception. In the case of views which at the present day have been definitely abandoned, I have only taken the *earlier literature* into consideration in so far as it possesses *historical interest*; otherwise I have contented myself with quoting from

those researches which actually *brought about* the definite decision. Unfortunately, it is not often that I have been able to apply this limitation, for the majority of the questions here touched upon still stand in need of a definite solution. And in these cases it has invariably been my endeavour to formulate the present state of the problem on the basis of *all* the investigations to which I had access. Yet I cannot but fear that there are gaps in my references, for the existing literature is very comprehensive, and is distributed throughout the most diverse branches of biology. I should, therefore, be very grateful to have my attention called to any work that I have overlooked.

In order to prevent the book attaining the proportions of a *hand-book*, I have condensed as far as possible all descriptions of *methods*, together with *figures*, *tables*, &c.; I also adhere to the principle that in the case of experimental researches it is absolutely essential to refer to the original publications, and hence regard a fuller description of methods as superfluous, unless *from one's own experience* they can be presented so concisely that they *are at least equal* to the original work in clearness.

The *references* have been taken almost exclusively from the *original publications*, so far as they were accessible to me in the *Erlangen library* and in the different *libraries of Berlin*. In other cases, I have usually given the place where I have found them quoted. In some instances I have not seen the *original*, and have forgotten the *place of quotation*; these have been distinguished by an asterisk.

CARL OPPENHEIMER.

ERLANGEN, *May*, 1901.

TRANSLATOR'S PREFACE.

AT the request of Dr. Oppenheimer I have introduced into the text the results of various investigations which have been made since the German edition of this book appeared.

The remarkable phenomenon of the reversibility of the action of a ferment, which was first recorded by Hill in the case of diastase, has recently been observed by Kastle and Loevenhart in their research on lipase, of which I have given an outline on p. 223. This confirmation of Hill's results will doubtless lead Dr. Oppenheimer to modify his view that a ferment can never effect a synthetic process.

The note on the *optimum* temperature for the action of acetic bacteria (p. 301) illustrates the way in which errors are copied from one text-book to another. From my personal observation, I can affirm that, in practice, acetic bacteria work well at a temperature far above their thermal death-point as given by many authorities. This is a striking instance of the adaptation of a ferment to its environment.

C. A. M.

57 CHANCERY LANE, W.C.,
September, 1901.

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FERMENTS.

CHAPTER I.

INTRODUCTION.

SINCE the time when their existence was first recognised, the subject of "Ferments and their Actions" has been the common ground of investigation in almost every branch of biology. Such processes, indeed, play an important part wherever life is found. All the problems of the transference of energy in animals or plants, or, in other words, of *life*, are in some way or other bound up with the doctrine of "Ferments." The phenomena, both of life and of enzymic action, manifest themselves in numerous ways, which, in many cases, are still obscure. But as yet there is, unfortunately, far from being any general agreement as to the significance and extent of the operations of either set of phenomena. Whilst, by many, fermentative processes are regarded as undoubtedly a part of vital phenomena, others deny this intimate connection, and assert that fermentative processes are only peculiar modifications of phenomena which are also occurring in the inorganic world. Nor, as regards the nature of ferments themselves, is there any certain and generally-accepted fundamental notion, any more than in the case of their relation to life. In fact, not even the definition of a "ferment" is settled.

It is, however, impossible to successfully attack a problem, so important to biology as the relation between ferments and life, before we have formed any notion of a "ferment" itself and a simple conception of fermentation. Now, in the course of centuries, this notion has conveyed various meanings. Its connotation has been frequently enlarged, narrowed, and again enlarged. But, in spite of all this, it was not until the present day that it became clear to some extent; though, even now, there is no actual agreement as to what is a fermentative process, or what phenomena it should include.

Perhaps, however, the right time has now arrived (and the work is well worth attempting) for the foundations of this comprehensive conception to be laid, specially since the latest experimental results seem likely to break down the barriers which have hitherto stood in the way, and to clear the ground for the erection of a new and higher scaffold, on which previously-observed facts may be built up by induction into a complete theory of *Ferments and their Action*. It would be presumptuous for me to assert that this higher scaffold has been reached in my comprehensive conception of a ferment, as set forth in the following pages; I am well aware that this conception has its weak places and gaps; yet I may surely hope that this attempt at a comprehensive survey offers at least a foundation on which more may be built.

If, now, we wish to obtain a comprehensive idea of the doctrine of ferments and their action, it is essential to make a survey of at least the main features of its historical development and of the notions which have been associated with it, so that, from a critical consideration of its numerous stages, we may attain our end.

The conception of *fermentatio* reaches back into the remote past. A knowledge of the practical significance of certain phenomena of fermentation, and especially of the *alcoholic fermentation of sugar* has been the common property of every race of mankind in every age. In consequence of this, the term "fermentation," which in this primary sense can almost be used as an equivalent for the German word *Gährung*¹ in its popular significance, has not, even in early times, been exclusively used by the philosophers. Nowhere, however, in the remote past do we find any scientific statement on this point.

Then, too, the confusion of the most different kinds of processes under this conception of fermentation soon commenced—a confusion which continued throughout the middle ages, and which, even at the present day, has not disappeared.

At one time the conception of *fermentatio* was commonly applied in its etymological sense² to all reactions which were accompanied by a visible evolution of gas, and to them only; at another, *putrefactio* (putrefaction) and *fermentatio* were used as completely synonymous terms.

This generalisation was still further extended, and an attempt was made to group all chemical processes under the universal

¹ Or English, "alcoholic fermentation."—Translator.

² It is probably connected with *fervere*, "to boil," "to seethe." (Cf. A. Mayer, *Gährungschemie*. Note, p. 7.)

heading of "fermentative actions," so that this conception afforded a crutch similar to that which, at a later period, was offered by "Catalysis." And, in addition to this, a secret vital force, the *lapis philosophorum*, also came to be connoted by the term "ferment," which made confusion worse confounded.

In the main, however, the term *fermentation* was restricted to those processes which were accompanied by an *evolution of gas*, so that HALES and VAN HELMONT¹ described even oxidations with nitric acid and the frothing of carbonates in acids as "fermentations."

This last result of regarding fermentative actions from a purely external point of view was, however, soon opposed by SYLVIVS DE LA BOË, who was the first to point out the fundamental difference between the liberation of carbon dioxide from carbonates and alcoholic fermentation.

Then LEMERY asserted that alcohol was not present until *after* fermentation, in opposition to the earlier theory of BASILIUS VALENTINUS, who had regarded the fermentation as merely a process of purifying the alcohol already present.

Then, too, BECHER discovered that the alcohol was first produced during the fermentation, and only from sweet substances; he drew analogies between fermentation and combustion, and distinguished between *fermentation* and *putrefaction*, and between true (*alcoholic*) fermentation and *acid* fermentation (formation of lactic acid, and so on). STAHL, the founder of the *phlogiston theory*, also studied the nature of fermentation.

He ascribed to the ferment an internal motion which it could convey by contact to quiescent substances, and thus cause them to undergo decomposition. A similar view was expressed by WILLIS. STAHL's theory was generally accepted. By "ferment," to speak in modern language, was understood a certain conveyer of force which decomposed and broke up the quiescent groups of atoms. Any chemical theories on the point were hardly to be expected, considering the state of knowledge at that time. Only a rough distinction was made between *alcoholic*, *acid*, and *putrefactive fermentation*. Such was the condition of affairs when LAVOISIER came upon the scene. His epoch-making work on the significance of oxygen for life, and his foundation of quantitative chemistry, necessarily completely revolutionised the theory of fermentations.

¹In treating of the early period I have followed, in the main, Kopp's monumental work *Geschichte der Chemie*, iv., 285 *et seq.* See also Schützenberger, *Die Gährungserscheinungen*, Internat. Wiss. Bibl., 1875, 10 *et seq.*

LAVOISIER definitely stated that alcoholic fermentation consisted of a *chemical transformation of the sugar into alcohol and carbon dioxide*. He further asserted that this process was reversible, and showed that the alcohol still contained oxygen.

The generation which, eager to experiment and construct, erected the structure of modern chemistry on the foundations laid by LAVOISIER, took no interest in a theme of natural philosophy so obscure as the "fermentative process" appeared. Even LAVOISIER took for granted the existence of such a "ferment," and studied only the chemistry of the *reaction*—the externally manifested *result*—without troubling himself as to the *cause*. And his immediate successors, and notably GAY-LUSSAC, did the same. Whilst the chemical process of alcoholic fermentation was eagerly investigated, the question of the "ferment" was practically neglected.

Of course, this is to be taken *cum grano salis*. We shall subsequently see, in the history of alcoholic fermentation, that many facts were brought together to explain the action of the yeast, but a *theory*, in the strict sense of the word, was not looked for. Nor can we regard as such the view of GAY-LUSSAC¹ that *oxygen* alone was the disruptive force in the transformation of sugar into alcohol, since, in the main, he too only believed that the ferment *originated through the medium of the oxygen*. This did not account for the *action* of the ferment when formed.

And to adopt the later view of BERZELIUS and MITSCHERLICH² that fermentation should be attributed to a *catalytic* or *contact action* is only giving a new name to an unknown quantity.

In addition to these, there were several isolated attempts to explain fermentation by chemical or electrical theories (e.g., MEISSNER, COHN),³ but these are of no importance.

In the meantime the circle of phenomena was considerably widened by the discovery of other organic substances which also exercised *contact actions* of the same kind.

ROBIQUET discovered in the kernels of bitter almonds an *albuminoid* substance which possessed the property of breaking up the *amygdalin*, also present, into hydrocyanic acid and sugar. This active principle was more closely examined by LIEBIG and WÖHLER and termed *emulsin* (cf. p. 209). Soon afterwards other agents of similar character were found : *Pepsin* by EBERLE

¹ Gay-Lussac, *Ann. d. Chim.*, lxxvi., 247, 1810.

² Mitscherlich, *Liebig's Ann.*, xvi. ; cf. *Berl. Acad. Sitzb.*, 1841, 379 ; 1842, 147.

³ Cf. Balling, *Gährungschemie*, 1845, i., 155 ; Mayer, *loc. cit.*

and SCHWANN; *trypsin* by CORVISART; *starch-dissolving diastase* by PAYEN and PERSOZ; &c.

These substances were also termed *ferments*, and regarded as *albuminous substances* which possessed the power of producing catalytic reactions. Simultaneously with the eager investigation of these phenomena, the theoretical side of the problems also came once more to the front, and the ground was prepared for a *theory of ferments* which should explain these different processes from a single standpoint.

Thus, then we arrive at the first actual *theory of fermentative action*, which embodies a comprehensive conception—viz., *Liebig's decomposition theory*,¹ which, although frequently modified by him in its details, always retained, as its germ, the idea that all fermentation must be regarded as a *process of disintegration* of the molecule ending in disruption and being set in motion by a *chemical decomposition* of the body which induced the fermentation. Thus, putrefaction was induced by albuminous substances which were themselves in a state of decomposition, alcoholic fermentation by the decomposition of the yeast, the decomposition of albumin with pepsin, by the breaking up of that albuminoid substance, &c.

We find then in Liebig's theory, for the first time since Stahl, a "dynamic" conception of the processes, and it has therefore special importance, even though its explanation of a disturbance of molecular equilibrium induced by a *chemical decomposition* proved quite untenable. A chemical decomposition of the ferments may be at once put aside. Neither the yeast nor the albuminoid substances undergo *any chemical change* themselves, either as a whole or in part, in the course of the metamorphoses which they effect. Moreover, the investigations of DUMAS² specially contributed to the refutation of Liebig's theory, since by them it was shown that fermentation was only set up by the most intimate contact between the ferment and its material, and, further, that other movements, notably the vibrations of sound, were without influence on the decomposition.

Liebig's theory was intended to apply to all fermentative processes, which it regarded as only special manifestations of a general catalytic process attended by decomposition; to the alcoholic and lactic fermentation of sugar as well as to the action of albuminoid substances.

Of the numerous examples which Liebig brought forward as

¹ Liebig, *e.g.*, in his *Ann.*, lx., 1; and in the *Chemischen Briefe*, 1865. Brief, 21; cf. also *Ann. Chem. Pharm.*, cliii., 1, 1870.

² Dumas, *Ann. d. Chim. et Phys.* (5), iii., 59, 1874.

analogies we need only refer to one which clearly elucidates his point of view: Platinum by itself is not attacked by nitric acid. If, however, an intimate alloy of platinum and silver be treated with nitric acid the silver dissolves and imparts its state of dissolution to the platinum, which now becomes equally soluble in the nitric acid.

In this way Liebig also wished to explain the decomposition of the ferments themselves, regarded as the cause of the decomposition of their materials.

When, shortly after, the classical researches of PASTEUR established the fact that alcoholic fermentation and several other fermentative processes were intimately connected with the vital activity of lower organisms it became customary to make a distinction between those processes which were due to the activity of *formed* or *organised* ferments, and those which acted without contact with living cells, and were termed *unorganised* ferments, and subsequently, after Kühne, *enzymes*, such as *pepsin*, *diastase*, *emulsin*, &c.

As regards the organised ferments Liebig's theory was forced into the background by the discovery of the important part played by micro-organisms in alcoholic fermentation, putrefaction, &c., and the more so since Liebig could not bring himself to admit the significance of the living cells. Hence, in the case of the *unorganised* ferments, also, the importance of his theory was not accepted. We shall have occasion to describe more fully in the history of alcoholic fermentation the development of this point; the purely biological conception of PASTEUR, that alcoholic fermentation was *life of the yeast without oxygen*, must obviously have proved a great obstruction to the formation of a general conception of a "ferment."

It involved indeed a complete primary separation of *organised ferments* from *enzymes*, and we have only innate conservatism to thank that a *formal* separation, which would have necessitated the abandonment of a simple definition of a ferment, was not at once decided upon, to which end attempts have been made frequently enough.

Pasteur's view that the phenomena of fermentation were to be ascribed solely to the metabolism of the micro-organism was soon accepted by the majority of experts. Of course the idea of the identity between enzymes and organised ferments had not been abandoned by everyone. In particular, BERTHELOT, TRAUBE, and HOPPE-SEYLER emphatically expressed the view that *even in living cells there were active enzymes*, perfectly comparable with those acting *outside* the cell and only differing

from them in the degree of their connection with the cell. Yet they did not succeed in *isolating* such enzymes, and this fact was continually urged against them by their opponents.

In this case, too, as we shall see later on, the truth lies in the mean of these two views. Pasteur was right in so far as that there are, in fact, many reactions, formerly known as *fermentative processes*, which can *only* be regarded *from a biological point of view* as being associated with the metabolism of microbes, whilst other reactions, of which Pasteur asserted the same, are *undoubtedly enzymic*.

Now although these views are of importance as regards the *relation of the two kinds of ferments*, they unfortunately offer no *theory of the action of ferments*. TRAUBE and HOPPE-SEYLER merely assumed a closer relationship between the two groups, without, however, making any attempt to explain their action. Even if Pasteur's purely biological *conception* were accepted as correct; yet his *theory*—that yeast withdraws from sugar part of its oxygen only in the absence of atmospheric oxygen, which then by "respiration" again appears as carbon dioxide, *i.e.*, that fermentation is *vie sans air*—would soon be rejected as false, since yeast also causes fermentation in the presence of oxygen.

Thus, then, although Liebig's theory of fermentation by communicated *decomposition* had been shown to be incorrect, no new theory had taken its place. A fresh attempt at this was made first by HÜFNER,¹ and then notably by NÆGELI² in his *moleculophysical theory*.

Naegeli took the important step of substituting *molecular vibrations* for Liebig's *chemical decomposition*. He assumed that the vibrations of the molecules, or of the atoms in a molecule, could be so *increased* by the *corresponding vibrations* of a second substance that, by mere contact with this substance, a disruption of the molecule of the first substance was brought about without the *catalytic* agent which induced the decomposition being itself affected. The slightest shock is sufficient to break up unstable molecules. This unstable equilibrium may be compared with the state of tension which exists in rapidly-cooled glass, which, at the lightest touch at a definite point, flies into a thousand fragments. Here, too, the slight blow is by no means the *cause*; it only brings about the disruption of a small aggregation of glass crystals, but in the act of separation these cause so great a shock to the neighbouring particles that these too are broken up, and so the small shock is the *effective momentum* for a great

¹ Hüfner, *J. pr. Ch.* (N.S.), x., 148, 385, 1874.

² Naegeli, *Theorie der Gährung*, Munich, 1879.

transmutation of energy. So, too, does the ferment act on the substance which is decomposed, since it leads to *new conditions of equilibrium* which involve chemical reactions in which it takes no part itself. Now, although this assumption does not *explain* exactly why *ferments* induce this disturbance—and we certainly cannot prove that they are possessed of specially active vibrations—yet at least we find in Naegeli's theory a *dynamic* conception of fermentative action as opposed to the biological view of Pasteur.

Naegeli, in fact, extended his theory of organised ferments in such wise that even living protoplasm could, in his view, emit special, active, atomic vibrations. He might, however, with the greatest ease have included *all* ferments in this elastic conception, and so have produced a comprehensive scheme of fermentative action.

Unfortunately he did not take this step. Although he was willing to explain enzymic action in an *analogous* manner, yet he regarded it as *completely* different from "vital" fermentation. He believed, too, that enzymes induced *endothermic* processes, to which conclusion he was led by the incorrectness of the earlier determinations of the heat of combustion of cane sugar. In short, Naegeli, in spite of his definite dynamic theory, firmly adhered to a belief in the *deep-seated essential difference* between unorganised and organised ferments. This antithesis of Naegeli's was due to the fact that enzymes acted *outside the cell*, whilst the phenomena of fermentation *only occurred in immediate contact with the living cell*. Further, he committed himself to the statement that *enzymes* had the power of converting substances which were insoluble and unsuitable for metabolism into *nutritives* capable of assimilation, whilst, on the other hand, organised ferments *lowered* the physiological value of these substances. However important this distinction may be for biological questions, it is absolutely unimportant in studying fermentative process from a *purely theoretical point of view*. We could equally bring forward *enzymes*, which also change good into inferior nutritives, and *organised* ferments, such as, for example, those which convert *cellulose into assimilable sugar*.¹ To sum up, Naegeli saw in this difference, on which he laid stress, only the *teleological expression of an essential difference*. The more deeply-seated cause of his differentiation was the

¹ As is probably done by many micro-organisms. As regards the other point, the products of the decomposition of albuminous bodies by *trypsin*, such as leucine, arginine, &c., are certainly not to be regarded as nutritive substances.

indissoluble connection of the one process with the living cell, whilst the enzymes could exert their action apart from the cell, although, unlike Pasteur, he did not *directly* identify the action of the organised ferments with their metabolism.

In that state of our knowledge it was then really very difficult to find room for a comprehensive conception of the notion of a ferment.

Moreover, LOEW'S¹ idea that enzymes are a *residue* which can store active force in the living protoplasm is much too vague to be capable of being closely examined. A similar theory was that of MEDWEDEW,² who represented the action of animal oxydases as a residuum of vital force. The views advanced by others (*e.g.*, A. MAYER³) were of a like character. With the radical notion of GAUTIER, in which direct vital properties are attributed to the ferments, we shall deal subsequently.

HANSEN⁴ was thus then in a certain sense justified in proposing to loosen the Gordian knot by simply severing the slight connection between organised and unorganised ferments—a connection which was only apparent in the historical development of the subject. He considered it best to let the idea of a ferment *entirely drop*, and to distinguish between the action of *enzymes*, which had no need of the living cell, and the sphere of fermentative phenomena which was to be regarded as a subordinate part of the *metabolism of the organisms*. A *dynamic* view of the fermentative processes was here abandoned, and the *biological* view brought into prominence as the basis of the conception. If it were not possible to bridge over the gap between *living* and *dead* ferments and to make room for a comprehensive dynamic conception, this view would be perfectly justifiable and of great service in elucidating the problems which present themselves.

But I hope to show in the following pages that this gap can be bridged over, and that it is quite possible to retain the actual notion of a ferment and to give it a comprehensive definition.

That the difference between the fermentative actions of enzymes and of living cells cannot be such a fundamental one is clearly shown by many facts which are too little taken into account in this connection. Thus it is quite impossible to draw a line of, indeed, only a hair's breadth between the enzymes given by the cell to the surrounding media and those other ferments which remain firmly attached to the cell. Whilst the

¹ Loew, *Pflüg. A.*, xxvii., 210. ² Medwedew, *Pflüg. A.*, lxx., 249, 1897.

³ A. Mayer, *Enzymologie*, Heidelberg, 1882.

⁴ Hansen, *Arbeiten aus dem botan. Institut. Würzburg*, iii.

healthy living cell *excretes* only certain enzymes, it firmly retains a series of other ferments. If, however, the cell be destroyed, or even its vitality weakened, a portion of these ferments passes into the surrounding media, and now, liberated from the cell, act as true enzymes (*e.g.*, yeast *invertase*). It is not easy to decide whether these ferments should be grouped with the *enzymes* or with the *organised* ferments, since under normal conditions they only act *within* the cell in the protoplasmic bond, but when *set free* they develop independent powers. And still more does this difficulty increase when we find enzymes which we cannot in any way *extract* from the cell, but which, nevertheless, act *independently of the vitality* of the cell, since they are still active when the vital energy of the cell is destroyed by substances poisonous to the protoplasm, as is the case with the *inverting ferment* of *Monilia candida*.

A further proof that fermentative activity is not identical with the vital process is afforded by the interesting observation of FIECHTER,¹ that although hydrocyanic acid completely destroys the *vital process* and *development* of yeast, it does not immediately check the *fermentative action* when a considerable amount of yeast is present.

On the other hand, DE BARY² asserts that the *Bacillus amylobacter* can be boiled for a short time (several minutes) with a solution of glucose without losing its power of developing, although losing its *fermentative* capacity. The same result can be obtained by cultivating it for several generations in media in which it cannot develop any fermentative activity. In like manner, many species of *Mucor* can be deprived of their powers of causing fermentation without their vital power being weakened.

In reviewing these facts, GREEN³ has made a fresh attempt to retain the general notion of a ferment, and whilst dropping, as was inevitable, the distinction between enzymes and organised ferments, he is now disposed to regard *all* fermentative actions as *vital processes*. This view appears to me unlikely to solve the problem.

In the first place, this purely biological view advances us no further in our knowledge of fermentations, whilst the radical separation of enzymes, as proposed by Hansen, clears a way in this narrower field, at least for a purely "energetic" point of view, by leaving *vital force* out of the question; the theory

¹ Fiechter, *Wirkg. d. Blausäure*, Diss. Basle, 1875.

² de Bary, *Vorlesg. üb. Bacterien*, Leipzig, p. 65.

³ Green, *Annals of Botany*, vii.

proposed by Green, on the other hand, throws the whole question back into the forbidden circle of "*vital force*."¹ But apart from the fact that this apparently simple conception carries us no further, and does not allow us to form any definite notion of a "ferment," since it even causes the actions of ferments to be immersed in the enigmatical depths of *vital processes*, it also suffers from other serious defects. It is surely going very far to always speak of the action of excreted enzymes as phenomena of the *vital process*, because they were *once* developed within the living cell, even when they exercise their activity *at a distance from, and completely unconnected with, their original source*. In this way one might arrive at the absurd conclusion that *strychnine poisoning* should be regarded as a kind of vital process of *nux vomica*, if the action of *pepsin* were represented as a vital process of animals. These enzymes are only connected with *life* in the fact that they are *products* of living cells, and are of considerable *biological* importance to *life*. The other weak point of Green's view is the absolute impossibility of classifying fermentative actions. We find, to still consider his view, ferments as vital phenomena of *higher* organisms (*enzymes*), and, according to the definition, completely lording it over the metabolism of *lower* organisms (phenomena of fermentation). But why draw the line at *mould-fungi*? If the metabolism of the *mould-fungi* is fermentative action, why is not also that of the *moss*? Why not also that of *all* higher forms of life? We should thus, by following up this purely biological principle, arrive at an *identification of the conception of a ferment with metabolism*, and should have given a new name to an old problem.² This objection is applicable not only to

¹ By "vital force" I mean here not that much-disputed *specific* energy of the vital processes, developing from all inorganic matter, but I conceive it purely *empirically* as the sum of the forms of energy, which compose "*life*," and the inter-action of which *has* not yet been explained, although it *may* possibly be explained on purely mechanical grounds. On the question of the justification of "*vitalism*" I take no side here.

² STOHRMANN (*Z. f. Biol.*, xxxi., 385) starts from another point in assuming that nutritive substances first enter into combination with the living protoplasm, and are then split off from the latter by means of *catalytic action* in the form of albumin, fat, &c. In this view, too, fermentative actions in the *widest sense* would have to be regarded as involved in the metabolism of the cell itself. This idea has been developed into a comprehensive theory by MAX KASSOWITZ in his *Allg. Biologie* (Vienna, 1899). (*Cf.* also my abstract on the subject in the *Naturwiss. Wochens.*, May 1899). It cannot, however, aid us in the consideration of the fermentative process by itself, since the processes which take place in the protoplasm do not admit of critical consideration.

Green's view, but in general to the idea of the action of organised ferments being the metabolism of lower organisms, as formulated by Pasteur. Nothing here justifies us in drawing an arbitrary boundary between the lower *mould-fungi*, whose metabolism is to constitute fermentative action, and *other* forms of life—*higher moulds*, chlorophyll-containing plants and animals. This has, moreover, been implicitly admitted by SCHÜTZENBERGER,¹ the most ardent supporter of the biological point of view, who explains the previous *differentiation of fermentative phenomena* as being due to defective knowledge.

The biological point of view is thus shown to afford no help in forming a comprehensive conception of a "ferment," and, if it be impossible to find a dynamic point of view and to bridge the gap between enzymes and organised ferments, we must fall back upon Hansen. We must distinguish between the lifeless *enzymes* and the *vital phenomena as such*; and it is then also justifiable within these limits to regard the vital phenomena of the lower *mould fungi* as separate from those of other organisms. The subject then becomes merged in purely *biological* questions which no longer concern the *theory of ferments*.

But there is no necessity for this. The facts mentioned above having already made the boundary line between organised and unorganised ferments indistinct, the epoch-making experiments of E. BUCHNER have rendered the distinction in its *theoretical* significance capable of removal. Buchner's success in isolating the *enzyme* of yeast cells, or *zymase*, the action of which had for so long been regarded as inseparable from their vital process, and in fermenting grape sugar by its means, independently of the cells, opens up the probability that the distinction generally between enzymes and organised ferments will disappear from the theoretical point of view.

This will furnish the indispensable preliminary condition for a comprehensive conception of a ferment, and this conception can only be of a "dynamic" character.

We must ask ourselves: *What must be the nature of the decompositions which we term fermentative actions; and at what point is the boundary between them and other processes to be fixed?*

Now, we know that the so-called *enzymes* have the power of resolving stored-up chemical tension; this has long been known as regards *alcoholic fermentation*.² All we have to do then is to regard by analogy *all* fermentative processes as *liberating*

¹ Schützenberger, *Die Gährungserscheinungen*, Internat. Wiss. Bibl., 1875, 1.

² Cf. also Stohmann, *Z. für Biol.*, xxxi., 385.

phenomena of the same kind, whether or no this liberating energy is more or less closely bound up with the protoplasm of the cell; on the other hand, we shall *separate* from fermentative actions every process in which phenomena other than the emission of energy take place, especially those in which a state of tension is *generated*.

We shall thus reduce the relationship of living cells to ferments to the fact that all ferments *originate* in living cells to the exclusion of similar factors in the inorganic world which also liberate energy; and that, biologically, ferments are an extremely important instrument of the organisms in which they are developed. On the other hand, the chief stress is to be laid upon the *dynamic* side of the question—the amount of heat rendered perceptible.

A chemical reaction in which the entire mass of the reacting substance takes up energy, as, for instance, the reduction of carbon to acetylene, can only take place with the addition of active force from outside; such reactions only occur at high temperatures or under the influence of the electric current. Generally speaking, if an endothermic reaction proceeds at the ordinary temperature without the addition of foreign energy, there must also be a parallel exothermic reaction which is numerically comparable with it, so that the sum total of the heat rendered perceptible must be equal to or greater than $+0$. When nitric acid is reduced by means of zinc to hydroxylamine and ammonia, with an accompanying absorption of heat, this endothermic reaction is counterbalanced by the exothermic solution and oxidation of the zinc, so that the total result represents an excess of energy. If, however, endothermic fermentative processes exist, they lack both of these necessary conditions. A specially active introduction of energy is not essential, since the actions of ferments proceed at low temperatures. And a reciprocal process *never* occurs. One has invariably to deal with reactions proceeding entirely in one direction, to which no thermo-chemically opposite reactions run parallel. If, then, we are unwilling to ascribe to ferments the capacity of concentrating like a concave mirror, free *energy* from somewhere or other, and utilising it to bring about endothermic fermentations, we must simply decide that *enzymic processes can only be exothermic*. Against such a daring conception of concentrations of energy by minute quantities of ferments, which, even in the process itself, appear to remain inactive, may be urged the fact that fermentative processes are *never* assisted, but are sometimes checked, by sunlight, in which surely such a concentration

would occur more readily. It is, therefore, at present not possible to conceive of an endothermic process being a fermentative process, since the source of the energy is unknown. On the other hand, *fermentative processes of an exothermic nature* are at once conceivable, since in this case the origin of the liberated energy is to be found in the molecular groups themselves, which are undergoing fermentation.

CHAPTER II.

ESTABLISHMENT OF THE CONCEPTION OF A
"FERMENT."

FROM these considerations we arrive at the *following definition* of the notion *ferment*.

A ferment is the material substratum of a peculiar form of energy, which is produced by living cells and adheres more or less firmly to them without having its activity bound up with the vital process as such; this energy is in a condition to bring about the liberation of latent (potential) energy of chemical substances and its conversion into kinetic energy (heat, light); in such manner that the chemical substance is so changed in the process, that the new substance or the sum of the new substances produced possesses a smaller potential energy (i.e., a smaller heat of combustion) than the original substance. In this process the ferment itself remains unchanged. It acts specifically—that is to say, every ferment exercises its activity exclusively on substances of well-defined structural and stereo-chemical composition. I will endeavour to elucidate the different heads of this definition.

I have described ferments as the *material substratum* of a form of energy, because we are still absolutely in the dark as to the chemical nature of ferments. Whilst on the one hand some are disposed to regard ferments as well-marked *chemical compounds* of definite composition, which have not yet been obtained in the pure state solely on account of the defective state of our knowledge, others consider them to be completely immaterial quantities of energy attaching themselves, as it were, to various chemical substances, like the electricity in a conductor (*Arthus*¹). The views fluctuate about the different intermediate points between these two extremes.

Formerly ferments were regarded as simply albuminous substances, or, conversely, fermentative activity was attributed to albuminous substances, as was especially asserted of the *diastatic* ferments (*q.v.*). On the other hand, solid, very active, preparations were obtained, especially from *pepsin* and *invertase* (*q.v.*),

¹ Arthus, *La nature des enzymes*, Paris, 1896 (Thesis).

which no longer gave proteid reactions and so lent support to the idea that chemical individuals were present. However, in spite of many investigations, no ferment has as yet been shown to be a simple substance with any sufficient degree of probability, and still less has the constitution of any been determined. Any opinion on this point must therefore be expressed with all reserve. We shall subsequently deal with this question more fully.

Of the ways and means by which these *peculiar forms of energy* develop their activity we have not the slightest trustworthy idea. We must simply resolve to regard fermentative actions as special phenomena of the ominous "catalytic" processes from which their differentiation is required by the fact that they are *produced by living cells*. *Catalytic action* is nothing more than a scheme of despair under which we may group chemical reactions which, while possessing a certain similarity in their course, cannot, without further knowledge, be explained by our simple chemical theories. With the advance of our knowledge, we have naturally been able to assign many phenomena which were formerly regarded as *catalytic* to simpler chemical laws, so that this useful idea has undergone a considerable limitation in its applicability. At the same time we must not forget that in its essence even the theory of simple chemical decompositions and of chemical *affinity* is, as regards our *theoretical knowledge*, only one vast enigma; that we have only been much longer accustomed to deal with these conceptions as indispensable fundamental axioms without being able to approach them otherwise than metaphysically, which also holds good in a still wider sense of the conceptions of matter and force in general.¹

It would thus, in fact, only be referring a smaller special enigma to the general enigma of chemical force if we succeeded in giving a purely chemical explanation of "catalytic" action, as Hufner has attempted to do in a very able manner. We shall subsequently deal with this again more at length, and also show that fermentative processes exhibit other very considerable differences from the simple catalytic processes.

In the meantime, in order to form a definite conception of these force-emitting agents we may accept Naegeli's view as a suitable representation.

¹ The danger which lies in regarding our scientific fundamental axioms not merely as *forms of view* but as *objective realities* in the metaphysical sense has been recently impressively referred to by one of the officers, BOLTZMANN, in his excellent speech before the Congress of the Munich Natural Philosophers, 1899 (cf. *Nat. Rdsch.*, 1899, No. 39, &c.).

Just as we are willing to regard the atoms in a molecule as being not at rest, but as vibrating in a state of equilibrium, so too we may also assume that there are also in the catalysing agents such vibrations, possibly of a very energetic nature, which by communication of their motion break down a system of stable tension in one place; and that now this disruption spreads spontaneously by virtue of the heat liberated at its commencement, without the catalysing agent being involved in the decomposition; the process first comes to a standstill on the restoration of a new and more stable equilibrium. This theory receives further support from the fact that the *modus* of fermentative decomposition processes is *similar*, although not analogous, to that developed by other agents of a purely chemical nature, as, for example, *dilute acids*; we shall go more fully into this question when discussing more closely the manner in which ferments act.

Another way of looking at the subject, which, however, also does not serve us as a mode of representation, is that the activity of ferments should be regarded not as remaining unchanged itself, but as being continually regenerated. Thus in the hydrolysis the ferment *itself* is said to first absorb water and then to *part* with it to the substratum which is undergoing the hydrolysis, being restored to its original condition to repeat this process. Apart from the fact that it is very difficult to conceive how the ferment in aqueous solution should be in a condition to again part with its water, and especially to substances (such as albuminous bodies) which otherwise have no affinity for water under these physical conditions, the theory explains nothing, for the question still stands on the same footing: *Why* does the ferment-hydrate, if I may so express myself, part with its water again to the substratum? It is evident that Naegeli's theory is a more simple and elastic conception than this somewhat artificial mode of representation.¹

Neither of them gives an *explanation*, but only describe in periphrases the everlasting *catalytic action*. In addition to these, the momentarily rapid action of individual ferments (*rennet*) has led an adherent of this mode of considering the subject (Fick)² to form a *catastrophic theory* of explanation, and for this reason to separate rennet from all other enzymes. An

¹ An attempt to determine experimentally by the increase in weight the amount of water which the ferment was said to absorb during the decomposition has given disappointing results. (A. Mayer, *Enzymologie*, Heidelberg, 1882, 107.)

² Fick, *Pflüg. Arch.*, xlv., 293.

attempt was made to explain decompositions attended with oxidation in the same way as hydrolytic decomposition. According to this the ferment was itself *alternately oxidised* and reduced, absorbing oxygen, and again parting with it to the medium. What has been said above also applies to this view. In this action the *organically-combined iron* has been thought to play an important part.¹

Another view, which appears to me more worthy of consideration is that which, proposed in outline by BUNSEN and HÜFNER, was supported by WURTZ in his research on papaïn. According to this view we are to assume that the ferment first combines with the substratum undergoing fermentation to form an unstable compound which rapidly decomposes, and that in the decomposition of this compound a decomposition of the substratum also sets in. This view receives support in particular from the facts that ferments, notably pepsin and papaïn, actually do enter into so stable a combination with fibrin that the components cannot be separated by mere washing. We shall not be able to draw the conclusions from these facts until later on, but we must at once assert in advance that a view which assumes a preliminary combination of ferment with substratum, although it allows of an elastic conception of the conditions of the beginning of fermentative action, is not suitable for development into a complete *theory of the action of ferments*. Whilst the inner nature of "fermentative" processes is an enigma, it is certainly not difficult to perceive their end and the external conditions of their course.

The essence of catalytic and of fermentative processes is embodied in the gradual, as exposed to the explosive and sudden liberation of accumulated tension, by means of those material substrata of energy, which for the inorganic world we describe as *catalysing agents*, and as *ferments* in the case of the organic world.² The ferment is as little the *cause* of the decompositions which it effects, as the electric spark in the eudiometer is the actual *cause* of the tremendous liberation of force in the combination of hydrogen and oxygen, but only the *liberating momentum*, or as the violent shock from a blow is the *cause* of the explosion of a nitro-glycerin cartridge; this conclusion may be drawn with certainty from the fact that the ferment is not injured in the liberation of pent-up stores of energy which according to its character it brings about, but remains intact. In fact, it only gives the impulse towards the conversion of unstable groups of

¹ Sacharoff, *Centralbl. f. Bakt.*, xxiv., 661, 1898; Spitzer, *Pflüg. A.*, lxvii.

² See Stohmann, *Z. f. Biol.*, xxxi., 385.

atoms into more stable groups, and this change, when once commenced, is complete without the further influence of external momenta.

From this view it necessarily follows that the new products formed in this way during fermentation must possess a smaller sum of potential energy than did the original material, since a part of the accumulated tension in the latter is liberated in the form of kinetic energy (especially *heat*). The products of every fermentation must therefore possess a smaller total *heat of combustion* than that of the original substance, inasmuch as this serves as a measure of the degree of tension still present. We see, then, that all fermentations must be *exothermic* processes, but that, on the other hand, we have no right to also describe as the actions of ferments chemical processes in which the *addition* of *external* energy is required, these being *endothermic* reactions in which the sum of the potential energy of the new bodies produced is *greater* than that of the original substance.

If we transfer this mode of reasoning from the general dynamic point of view to chemical processes, we see that within the limitations which hold good for fermentative processes, in which a simple exchange of atomic groups of different tension (*substitution*) does not occur, only *two* chemical reactions can, without straining the point, be attributed to fermentative actions, viz.:—In the first place, decomposition accompanied by an absorption of the elements of water—*hydrolytic decomposition* analogous to that effected by acids or alkalies; and secondly, oxidation, usually in conjunction with a separation of the molecules—*oxidising decomposition*—in which atmospheric oxygen may be utilised, or which may proceed without such assistance, as in *inner oxidation* (*vide infra*). On the other hand, according to our view, it is not permissible to assign other processes, such as reactions of a *reductive* or *synthetic* character, to fermentative actions.

It appears to me urgently necessary to postulate this limitation to the nature of fermentative processes, because, owing to the close relationship in which ferments stand to the vital process, specially in the case of lower organisms, it is only possible by means of this strict differentiation to draw a firm line of demarcation between fermentative processes which are caused by living cells, and *purely biochemical* decompositions, which are actually a condition of the metabolism of the living organism, and inseparably bound up with it. We must, therefore, learn to distinguish between the *ferments* of *living cells*, whose activity we can conceive of being separated from the cell, and which

indeed can also in many cases be *de facto* so liberated, and the vital processes of the cell as such, for which naturally reductive and synthetic *endothermic* processes are also admissible. Fermentative actions, or at least an important part of them, must no longer be identified with the metabolism of certain lower organisms. *Fermentation* phenomena are, as we shall see later, a mixture of processes, which are either indisputably fermentative, or can at least be regarded as such without straining the point, with other processes in which reductive and synthetic reactions take part, and which are only produced by the purely vital metabolism of these organisms. For these reasons¹ it is perhaps best to throw overboard altogether the notion of *fermentation* (Gährung) as it has developed historically, and to draw a distinction between the *fermentative actions* of lower organisms which are to be placed in complete analogy to those of higher animals and plants, and the *biochemistry* of micro-organisms in the narrower sense, which by imperceptible stages, nowhere sharply differentiated, approaches that of the higher organisms. If we do not resolve to completely separate chemical processes of an *endothermic* character in the lower forms of life from the fermentative processes in the same organisms, it is absolutely impossible to keep within definite bounds the subject of the *action of ferments*.

Processes such as, for example, the transformation of atmospheric nitrogen into ammonium salts, of sugar into *mannite*, of formic acid into marsh-gas, and the reduction of sulphur compounds by varieties of *Beggiatoa* (Cohn),² which are brought about by micro-organisms, are the sort of processes which *must be separated from fermentative processes and assigned to the biochemistry of the micro-organisms*. We therefore no longer draw the boundary line between organised and unorganised ferments, but between ferments in general in the sense given above, and the *vital process* as such, the dynamic decompositions of which are subject to no limitations, since it possesses a source of energy in its own protoplasm; we no longer differentiate in accordance with a *biological*, but with a *dynamic* principle.

The reason why the fermentative processes have been brought into so close a connection with the metabolism of the lower organisms is, as we have already explained, due to the fact

¹ Of course, this is *only* for this theoretical point of view. In practice, the terminology "fermentation" (Gährung) is too valuable to us as a stop gap, at least, to be discarded.

² Cohn after Pfeffer, *Pflanzenphysiologie*, Leipzig, 1881, 369.

that until recently many processes of undoubtedly fermentative character were looked upon as *inseparable* from the living cell. In this sense a distinction was made between the ferments which could develop their activity independently of the living cell, and were termed *unorganised ferments*, or, in accordance with KÜHNE's suggestion, *enzymes*, and the *formed* or *organised ferments* which were only active in immediate contact with the living cell. In the wider sense, too, the bearers of these ferments, the cells, were themselves termed organised ferments.

We have, however, shown that there is no essential difference in the nature of unorganised and organised ferments, but that the only quantitative difference between them lies in the way in which they are more or less closely connected with the living protoplasm. And if, from the facts hitherto observed which point in that direction, we venture to draw the conclusion that *all* ferments act, in the strictest sense, *independently* of the vitality of the cell, and that it is possible with more or less difficulty to *establish* this independence, the barrier between unorganised and organised ferments will vanish in favour of a uniform method of viewing *all* true fermentative actions, even though, as indeed has *hitherto* actually been the case, there are certain processes of the kind which cannot yet be separated from the living protoplasm, or even if this separation should not be accomplished in the future.

All ferments have in common the fact that they are *produced* by living cells, but there are considerable differences in the relationship in which they stand to the mother cell after their formation. We have seen that the *degree* of this connection varies from that of the simply *excreted* ferments to that of those which hitherto it has been absolutely impossible to separate from the living cells (*lactic acid fermentation*). But, for our point of view, it is not a matter of decided importance whether the activity of a ferment can actually be *observed* apart from the vital activity of the cell which produced it; it is sufficient to form a *conception* of its activity under these conditions.

We will then emancipate ourselves, theoretically at least, from this distinction between *organised ferments* and *enzymes*, and term all processes fermentative in which, by means of living cells or their products, such *exothermic* processes of decomposition are brought about. On the other hand, we will separate from fermentative action all processes in which the living cell, by virtue of the energy contributed to it from outside, accomplishes *endothermic* processes, such as those of reduction and synthesis, because, according to our view, such processes, be-

longing exclusively to the vital process as such, must be relegated to another branch of science—the *biochemistry of the protoplasm* in the narrower sense. This theory includes the biochemistry of the *higher* organisms as much as that of the *lower*; the synthesis of carbohydrates and albuminous substances, of the alkaloids and the colouring and bitter principles of the higher plants, as well as that of the glycogen, hippuric acid, urea, &c., of the higher animals; and we will consistently refuse to group such processes under fermentative actions. In this sense, then, we can as little recognise a *urea-forming ferment* of the liver¹ as a *reducing ferment* of the tissue; or as little as one would agree to the assumption of a *strychnine-forming ferment* in species of *strychnos*, or of an *inulin-forming ferment* in dahlia seeds.

Here I must still dwell upon a primary point of great importance. Theoretically, it would be possible as a result of the purely dynamic view of fermentative processes to go so far as to effect a primary separation of *all* exothermic processes of the living cell from the endothermic processes, grouping the former in their entirety as *fermentative processes* on the one hand, and the latter on the other as *specific vital processes*. We should then, also, describe as fermentative the far-reaching processes of combustion in living cells, which lead to the formation of end-products no longer possessing *any* tension, such as, notably, *carbonic acid*, *water*, and salts saturated *ad maximum* with oxygen (*e.g.*, *sulphates* and *phosphates*). I should not, however, care to draw this radical conclusion. Although I must regard all fermentative processes as exothermic, yet all exothermic processes of organisms need not therefore of necessity be of a fermentative character. We must endeavour to preserve the historical conception, as far as is possible at least, and not run the risk of bringing incompatible things under a common notion by reason of too far-reaching a generalisation, and thus possibly creating fresh confusion. These complete oxidations in which the living cell applies to its own use the tension of so *active* a substance, while it saturates all its affinities with oxygen, are surely essentially different from the fermentative processes, in which, with a loss of energy, more stable conditions of equili-

¹ Of course, this only holds good in so far as the formation of urea in the liver is, in accordance with common conception, a *synthetic* process (from ammonium carbonate); but not as an explanation of the *splitting off* of urea from larger molecular groups, which may be accomplished by an *enzyme of the liver* (Richet, *Compt. Rend.*, cxviii., 1127). (For particulars, see *Urea ferment* in the special part.

brium, which are also still in a state of tension, are substituted for conditions of unstable equilibrium. The fermentative *modus* need not be the only one to which the living cell has recourse to disintegrate substances of high molecularity. We are as little compelled to draw an analogy between the ceaseless *combustion* of carbohydrates in the organism and the fermentative compositions as to admit an analogy between the decomposition of starch by dilute acids and its complete oxidation to carbon dioxide and water by glowing copper oxide or potassium bichromate and sulphuric acid. Although it is, theoretically, not completely out of the question to show that, *in reality*, all such processes may be brought about indirectly (*i.e.*, by ferments), yet such an assumption, for which it is quite impossible to find any experimental proofs, is too contrary to our conception to enable us to draw such an analogy between simple decompositions and radical combustions.

Whether this "combustion" be regarded as intramolecular respiration after Pflüger, or the catastrophic hypothesis of Kassowitz be adopted—in either case it would, in my opinion, be going too far to describe without further proof these oxidation phenomena as fermentative processes, merely because they too are *exothermic*. We will content ourselves with having sought for a comprehensive definition of the fermentative process without attempting now to explain *all* the exothermic reactions in the vital process. Yet, simple as is this purely dynamic comprehensive conception from a theoretical point of view, there are still some difficulties when we now proceed to assign, on the basis of this conception, definite limitations to the material with which we are to deal.

Although, as I have already shown above, much of it may at once be excluded, in which without doubt *endothermic* processes appear either alone or in a marked preponderance, whilst another part may be as readily included; yet there remains a not insignificant residue, with which, in the absence of any fixed theoretical basis, we must deal more or less arbitrarily.

This holds good, for instance, for the manifold and innumerable decompositions which are effected by means of *micro-organisms*. Many of these are pure decompositions, and as such *exothermic*—*i.e.*, fermentative processes, as, for example, the decomposition of grape sugar into *lactic acid*, which, indeed, is also produced from the sugar by a regular chemical decomposition; likewise the dissolving of albuminous substances, and of gelatin by bacterial ferments, some of which can even be isolated.

Other bacterial actions must without further consideration be separated from fermentative processes as being purely metabolic products, such as, *e.g.*, the production of tubercle, fat, &c., in media containing no albumin, in which, undoubtedly, *synthetical processes* take place.

It is more difficult to come to a decision about numerous other more complicated cases, as the circumstances compel us to definitely assign the processes to *metabolism*, or else to assume the presence of many as yet unknown ferments in one and the same organism. A typical example may serve to show whether I am justified in omitting all these peculiar bacterial decompositions, although possibly part of the processes is of a fermentative character.

According to GRIMBERT,¹ *Friedländer's pneumobacillus* produces from *mannite* much *lactic acid*, together with much *acetic acid* and *alcohol*; from *dulcite* it produces much *succinic acid*, no *lactic acid*, and but little *acetic acid*, with much *alcohol*; and from *arabinose*, much *lactic acid*, no *succinic acid*, much *acetic acid*, but no *alcohol*; with *xylose*, again, it acts differently. Other micro-organisms behave in a similar manner. Although, as I have said, I will readily admit that fermentative processes actually do also *take part* in these phenomena, yet it appears to me disadvantageous on *practical grounds* to discuss all these processes in a treatise on ferments. *Practically* they belong to the *biochemistry* of micro-organisms.

For similar reasons I have decided to omit here *putrefactive processes* in the narrower sense. Although true fermentative processes without doubt take part in these decompositions (*e.g.*, of cellulose, glycerin, albuminous substances, and the like) as effected by putrefactive micro-organisms, yet they are so interwoven with other processes, doubtless of a purely biochemical reductive nature, that it is impossible to isolate and to group them with the fermentative processes. Although in putrefaction typical hydrolytic decomposition products are formed from proteids by genuine fermentation, yet for the most part they are changed subsequently by the purely biochemical processes of reduction and synthesis. Thus indol derivatives are probably formed by synthesis and reduction from amido-acids; but certainly the *putrefactive bases* (such as, for example, *putrescine* and *cadaverine*),² *fatty acids*, and *hydrogen sulphide* are formed by reduction; and, in an analogous manner, *methane*

¹ Grimbert, *C. R. Soc. Biol.*, xlviii., 191.

² Ellinger (*Ber. d. d. chem. Ges.*, xxxii., 1899) has succeeded in splitting off these bases from ornithine and lysine by means of putrefaction.

from cellulose. In any case it is impossible, out of this extraordinarily complicated association of the most diverse chemical reactions, to form a conception of a true fermentative process which can be regarded independently of the vital process, and no Buchner will succeed in isolating from the bacteria a *putrefactive enzyme* with its typical capacities.

The so-called *butyric acid fermentation* appears to me to stand upon the same footing. In so far as it can be attributed without doubt to a reduction of *oxy-acids* caused by micro-organisms, it is certainly *not* a fermentative process. This is probably also the general rule, even when the butyric acid is formed directly from sugar; in any case the process is so far from being uniform, since every kind of micro-organism and every material lead to the formation of different products, that it is impossible to look for a true fermentative process in the medley of biological decompositions. I therefore refrain from discussing it, as also the *viscous fermentation* and other decompositions of carbohydrates due to micro-organisms.

CHAPTER III.

THE CHEMICAL NATURE OF FERMENTS.

IN the first place, as regards the "organised" ferments, this question may be dismissed in a few words. As constituents of the protoplasm, they are, as a rule, not accessible to chemical investigation. Even Buchner's zymase has not been isolated in such a state that we can think of a thorough chemical examination. It appears, however, to stand in close relationship, at least, to the albuminous substances.

This also is the case, in the main, with the *enzymes*. Whilst at the present time, many, especially French investigators (ARTHUS), regard *enzymes* as *immaterial* centres of energy which are bound to a sub-stratum, the nature of which has no bearing on their activity, and which should even be in a condition to bring about reactions from a *distance*, yet the majority look upon enzymes as definite *chemical compounds*.¹

But in spite of long controversy no decision has yet been arrived at as to the nature of these substances.

The difficulties of such investigations, already great in themselves, have been increased tenfold by the fact that it is exceedingly difficult to free ferments from foreign substances. Hence it happens that, notwithstanding all the labour expended upon this subject, no ferment has up to the present time been prepared in even an approximately pure condition.

¹ Compare with this the very singular idea of DE JAGER (*Virch. A.*, cxxi., 182, 1890), who maintains the complete immateriality of ferments, and now in support of his theory asserts that he has observed ferments acting at a distance through the medium of ether (*i.e.*, in the *air*!), as also the spontaneous formation of substances causing fermentation. Such a theory was specially adopted for *rennet* by FICK (*Pflüg. Arch.*, xlv., 293, 1889), who found that when a layer of milk was carefully placed over an active solution of the ferment, the whole of it *immediately* coagulated, notwithstanding the fact that the upper parts of the layer did not come so rapidly into contact with the ferment; this interesting observation was, however, contradicted by LATSCHENBERGER (*C. f. Phys.*, iv., No. 1, 1890) and LEA and DICKINSON (*J. of Physiol.*, xi., 307), who showed that if a mixture were *really* prevented, a coagulation of the upper layers only occurred after several hours (*cf.* also WALTHER, *Pflüg. A.*, xlviii., 529).

Ferments have the property of being withdrawn from their solution by falling precipitates, which specially increases the difficulty of separating them from albuminous substances. Moreover, the temperature throughout all the operations must never exceed 70° C., since with that degree of heat ferments become inactive, and all possibility of recognising them is then lost.

It is then not surprising that even at the present time not even the first question whether ferments should be regarded as *albuminous substances* has been satisfactorily answered.

Earlier observers (LIEBIG, ROUBIQUET, &c.) simply considered ferments to be "albuminoid substances."

Afterwards many investigators very energetically opposed this view—particularly HÜFNER, in the case of *pancreatin* (*trypsin*), WURTZ in the case of *papaïn*, and BARTH and ZULKOWSKI in that of *diastase*. They based their views principally on the wide difference between the results of their analyses and those of albuminous substances.

At the present time, however, all these earlier researches are not to the point, since it is abundantly clear that these investigators had in their hands extremely impure preparations partially contaminated, in particular, with a large proportion of higher carbohydrates, so that their analytical results are without value. For this reason I also reject their conclusions.¹

LOEW,² too, had no difficulty in proving the unreliability of all previous investigations when he propounded his theory that ferments were different varieties of "active peptones."

His criticism, which was fully justified, received special support from his proof that the saccharifying ferments were invariably contaminated with insoluble carbohydrates—viz., gums and dextrans—which must have had a disturbing influence, not only on the results of the analysis, but also on the investigation of the decomposition products. Thus, Loew concluded that the fact urged by Barth as a proof against the albuminous nature of *invertase*—viz., that he could find no *leucine* after decomposition with sulphuric acid—was accounted for by the fact that any *leucine* present could not crystallise out from the sugary syrup which was formed. He himself obtained from *diastase* a purer preparation, which gave the reactions of peptones (or, according to our present views, of albumoses).

He assumed that unorganised ferments were formed from the protoplasmic albumin by a process of *depolymerisation*, in which,

¹ A table will be found in Loew's communication, *Pflüg. A.*, xxvii., 204.

² Loew, *Pflüg. A.*, xxvii., 203, 1882.

out of about twelve aldehydic groups, which, according to his view, protoplasmic albumin contained, only two or three still remained attached. He then attempted to prove the presence of free aldehydic groups in the enzymes, and showed that active pancreas ferment gave an intense *black coloration* with neutral silver oxide, whilst boiled (*i.e.*, inactive) solutions of enzymes only showed a very faint brown colour under the same conditions.

This very interesting fact constitutes the only proof of a chemical difference between active and inactive ferments. It has only once been entirely confirmed, and then in the case of glycerin extracts of vibrios, specially of cholera, by MACFADYEN¹ who also adheres in other particulars to Loew's view that ferments are "unstable" albuminous bodies. They are, moreover, to be thus classified, he argues, from the fact that they contain *amido-groups*, which LOEW² endeavours to show is probable, inasmuch as they are rendered inactive by formaldehyde. This simultaneous presence of aldehydic and amido groups should necessitate their instability. Yet the ferments are certainly not unstable, but *stable*, and only bring about the disruption of *other* unstable (*potentially unstable*, according to Loew) molecules; indeed there is some reason for assuming that ferments before acting attach themselves to the *substrata*, in which process intermediate changes in their own structure may well take place. The whole conditions, however, are far too obscure for Loew's view to be accepted with success as a "heuristic"³ principle. As regards the original issue of the theoretical view—the acceptance of the albuminous nature of ferments—Loew's experiments have not brought the problem nearer to solution. That active ferments give albumin reactions only proves that even carefully purified solutions of ferments still *contain* albuminous bodies; it cannot, however, be inferred from this that these albuminous substances are the ferment *itself*.

In fact, by repeating tedious processes of purification, very active preparations have been obtained, especially of *pepsin* and *invertase*, which no longer gave any proteid reactions. We can easily dispose of the oft-repeated objection⁴ that the ferment

¹ Macfadyen, *Journ. of Anatomy and Physiology*, xxvi., 409, 1892.

² Loew, *Science*, 1899, p. 855. He also refers to a similar proof given by NENCKI.

³ A "heuristic" principle (εὕρισκειν = to find) is a hypothesis or view which not only summarises known phenomena, but which, being accepted, affords premises for further conclusions. It has practically the same significance as "working hypothesis."

⁴ Cf. *inter alios*. Pekelharing, *Z. physiol. Chem.*, xxii.

itself in this case may still be an albuminous body, which is only present in so small a quantity that the ordinary reactions do not take place, although the ferment is still active in this extreme state of dilution. We have before us a dry substance which dissolves readily in water, no longer gives proteid reactions, and contains only insignificant quantities of ash and carbohydrates; here then we have a chemical substance of a characteristic nature, which is either the ferment itself, or is attached to the ferment; surely in either case, under such conditions, the ferment is not an *albuminous substance*.

However, this does not hold good without qualification for all enzymes. In fact the latest investigations on *diastase* (WRÓBLEWSKI) seem to prove that pure diastase is a substance of an albumose nature, and it is not possible to absolutely free *trypsin* from its albuminous characteristics.

The unsatisfactory part of all these investigations is always the fact that ferments can only be recognised by their specific action; and that they do not respond to any chemical test. For certain colour reactions as, for example, with orcin and hydrochloric acid (*vide Emulsin*) are so uncertain and most probably to be attributed to impurities, that it is not worth bringing them forward as in any way general reactions for enzymes. Hence the constitution of enzymes is still at the present day one great enigma. For we do not possess more exact chemical results even in the case of enzymes apparently free from albumin.

More recently the view has been expressed in several quarters that ferments possess a more complicated structure than albuminous bodies in the strictest sense.

KÜHNE had previously announced that on heating, or on treatment with acids, an albuminous substance was split off from trypsin. Hence he ascribed a very complicated structure to trypsin.

Then PEKELHARING¹ was the first to conceive the idea that pepsin might be a *nucleo-proteid*. He prepared from gastric juice a very active substance, which he regarded as a very pure pepsin, and this on decomposition yielded, in addition to a substance of an albuminous character, *xanthic bases*, but apparently no carbohydrate.

SPITZER, too, claimed for a part, at least, of the oxydases of the animal body, the constitution of nucleo-proteids containing phosphorus and iron.

FRIEDENTHAL² next separated from the perfectly pure gastric juice of dogs, which was obtained by means of a fistula, only one

¹ Pekelharing, *Z. physiol. Ch.*, xxii., 233.

² Friedenthal, *Arch. f. Anat. u. Phys. (Ph. A.)*, 1900, 181.

albuminous substance, which he precipitated from an acid solution. This yielded xanthic bases on decomposition with concentrated hydrochloric acid, gave the colour reaction of pentoses, and contained phosphorus and iron. He could obtain this substance by "salting out" with ammonium sulphate from commercial preparations of pepsin as readily as from this gastric juice. He came to the conclusion that it was a substance of a nucleo-proteid character with a very complicated structure.

He obtained similar preparations from commercial *diastase* by salting them out with common salt from a solution slightly acidified with acetic acid; but, unlike Wróblewski, he found no other albuminous bodies. *Trypsin* (RIEDEL) and *papayotin* (RIEDEL) also yielded, when subjected to the same treatment, nucleo-proteids, which Friedenthal regarded as the actual ferments. It should, however, be noted that his diastase preparation yielded *only* 0.76 per cent. of *nucleo-proteid*. Moreover, since he refused to allow to Wróblewski's *Araban* (cf. under *Diastase*) any considerable share in the composition of the diastase preparations, and yet could find no other albuminous substance in it, the question naturally occurs: Of what did the remaining approximately 99 per cent. of the dry preparation consist? Surely the residue unaccounted for could not have consisted *exclusively* of ash or glucose, which he undoubtedly found in large quantity in the preparation. We are as yet far from a definite solution of the problem of the nature of ferments, as indeed Friedenthal himself admits in concluding his interesting investigation. MORACZEWSKI¹ regards the enzymes as "decomposition products of the substances on which they act specifically," and believes that a fundamental importance may be ascribed to the *calcium salts* which they contain; what he exactly means is not quite clear to me; he himself admits that he has *no proof* in support of his views.

The great difficulty of isolating fermentative substances, and the peculiar nature of their actions, but specially the great significance which they have for the vital process, have led certain investigators to the opinion that ferments stand materially in a very much closer relationship to the protoplasm than is commonly accepted. The majority express themselves not less cautiously than vaguely that there are in ferments "fragments of protoplasm" endowed with "residues of vital force," or the like.² But nothing can be made of such expres-

¹ Moraczewski, *Pflüg. A.*, lxi., 32, 1898.

² Armand Gautier, quoted by Effront, *Les Diastases*, loc. cit. The original was not accessible to me.

sions, which convey absolutely no idea, and which are completely removed from an experimental basis, since they evade all criticism. As a *heuristic* principle they lead to nothing. ARMAND GAUTIER has followed up this remarkable view with the inference that not only are the "residues" of vital forces to be assigned to ferments, but a considerable proportion of those forces, since he ascribes to them fundamental phenomena of the cells—viz., assimilation and reproduction. He thus to a certain extent regards ferments as *dissolved cells*. This hypothesis, which is based on a single experiment, is opposed to all that has been found by tedious experimental work with reference to the differences in the results of external influence on living cells and ferments. Unless Gautier still has very important facts in support of his view *in petto*, we must certainly put it aside as a curiosity; it is evident, however, whether a logical development of the idea of "protoplasmic fragments" leads. It offers, of course, a mode of representation which, although not specially belonging to our subject, leaves open the possibility that there may *actually* be *dissolved cells*—i.e., vital forces in unorganised media. I allude to the *mosaic disease* of the tobacco plant discovered by Beyerinck,¹ which is undoubtedly a contagious affection, notwithstanding the fact that Beyerinck could not discover any micro-organisms as its cause, and which, according to his view, is communicated by means of a *contagium vivum*, a contagion which can be precipitated by alcohol without losing its activity. It seems, however, from this that we are dealing with an *intoxication* rather than an *infection*. The whole subject is still very obscure.

Some observers, indeed, have found micro-organisms, and, on the other hand, these may have escaped detection by reason of their extraordinary minuteness or other characteristics.

Indeed, we do not even know the cause of such undoubtedly contagious diseases as scarlet fever, smallpox, and syphilis. It is certainly striking that all these hypothetical, extremely small micro-organisms absolutely refuse to develop on any known culture-medium. The *possibility* is not out of the question that, by reason of such "liquid contagia," which are possibly also the cause of the human diseases mentioned above, our conception of the "cell" may undergo such a radical change as to admit that vital forces can actually act in unorganised media; and in such a case Gautier's view would be justified. But not until then can it be subjected to serious criticism as a whole. But in any case, having regard to the *possibility* of such dissolved vital centres, it

¹ Beyerinck, *C. f. Bakt.* (ii.), 1899, 27.

is incomparably more important than the untenable talk of the "residues" of vital forces. Ferments are either *vital* or they are not. No compromise is possible. In the meantime, however, we must adhere to their separability from life.

We must next consider the conception that ferments in general are not material substances, but only properties of substances—an idea which has been supported in a very ingenious fashion by ARTHUS.¹ After showing irrefutably that attempts to prepare in a pure state, and to chemically individualise, ferments have not led to any conclusive result, he draws a parallel between simple physical forces and enzymes. Just as light, heat, electricity, &c., were at first regarded as substances, so it has been with ferments, and he expresses the hope that like them, as our knowledge advances, ferments will be erased from the list of *material substances* and be classified with the imponderable forces.

He argues with complete justification that material properties ascribed to the ferments can also be assigned to forces; he then shows that just as heat destroys the activity of ferments, so too, although at a higher temperature, it destroys magnetism, and so on.

It is very difficult to follow to its conclusions this idea of the immateriality of ferments; still more difficult to oppose it with arguments of exact science. For here we are dealing with a difference of opinion, which, in reality, is only apparent, and disappears when regarded from the theoretical standpoint of view.

If we take our stand on the basis of the dynamic conception of the universe, which altogether denies objective reality to matter and is exclusively concerned with the relationship of forces, this conception of the ferments is readily intelligible; where universal matter consists only of centres of energy, there is no room for a material conception of ferments.

The question now arises as to how far this method of considering the question within the dualistic point of view can lead to any results.

In this view, matter is only a metaphysical postulate; whatever *properties* of matter come subjectively to our consciousness by the aid of our senses are, without exception, perceptions of the energetic rays of matter, in accordance with which we formulate our theories.

We cannot even conceive of the action of light, heat, or electricity, otherwise than by representing them to ourselves as

¹ Arthus, *La Nature des Enzymes*, Thèse. Paris, 1896.

effective forces in material substrata. We conceive heat as the vibrations of material molecules, sound as vibrations of the air; even in the case of light and electricity we have created an imaginary something—the hypothetical ether. In this sense, too, we cannot conceive of ferments as apart from some form or other of material substratum. If we follow this conception further, we arrive at a comparison with those phenomena which come nearest to the fermentative actions—the manifestations of *chemical energy*. It is strange that Arthus has not devoted more attention to the relationship of fermentative actions to simple chemical processes from a theoretical point of view. Chemical force stands in the most intimate connection with other forms of energy, with which it can exchange itself in a variety of ways. Where chemical energy (state of tension) is formed, other forms of energy (light and heat) disappear, and where chemical tension is resolved other forms of energy are set free, and yet, especially as a means of considering it, it is entirely bound up with a material substratum. When, for example, we speak of sulphuric acid, which we would surely regard as a material substance, in reality we never speak of anything but the *dynamic relations* of this *chemical substratum*. We know that this substratum has a special taste—emission of a characteristic form of energy on to the perceptive organs of taste—that it combines with bases, expels other acids from their salts, &c.: all dynamic conceptions which are nevertheless for our method of thought firmly bound up with the material conception—sulphuric acid. A sulphuric acid reaction without sulphuric acid is empirically inconceivable to us. And we know further that the substratum of energy which we term sulphuric acid has the power of producing phenomena, which other similar substrata of energy are unable to bring about. And, therefore, we assume for this dynamic individuality a material individuality also, and name this material substratum, in empirical contradistinction to all other material substrata, “sulphuric acid.”

The case of enzymes, *ferments*, is analogous. Assuming that there were an absolutely pure *pepsin* freed from all foreign substances, we could only individualise this material substratum by the fact that we found in it specific emissions of energy peculiar to it alone, such as, for example, the capacity of causing the decomposition of albuminous substances. We should then, as in the case of sulphuric acid, draw the conclusion of a material individuality from these specific manifestations of energy, and should describe this substance *empirically* as a chemical individual—as material pepsin.

We can thus form a conception of a ferment as an active principle as readily as of simple sulphuric acid; there is no ground for also empirically separating this special dynamic activity from the notion of matter, as theoretically it must be separated; such a conception would merely lead to a confusion of our crude mental idea of a *chemical substance* with the metaphysical relation between matter and energy.

Even as a *heuristic* principle, apart from its metaphysical untenability, this method of considering the subject will not lead us further. The question assumes a somewhat different form when we regard the problem from a purely empirical point of view. It is then no longer: Are ferments substances or properties of substances? but becomes: Are the material substrata, to which we believe the fermentative activities to be attached, *definite* "chemical substances" for each individual ferment, or can this form of energy serve as a substratum for *different kinds* of substances?

This question will presumably be experimentally answered. In the meantime certain considerations can be brought forward, which, *a priori*, make it probable that ferments, materially considered, are really *substances*. When, for example, we assume that diastase is bound up with an albuminous substance, it follows that, since this albuminous substance manifests the property of dissolving starch *in addition to* the ordinary proteid reactions, it differs in some way *materially* from other albuminous substances which do not possess this capacity. It is, therefore, also *materially* a *chemical individual*, for the same reason that we separate from one another as *chemical* individuals two kinds of sugar of otherwise similar nature, which show totally different rotations of polarised light, although they are only distinguished by a purely *physical* property. Just as we assume that such differences are the result of atomic groupings of a particular kind, so, too, we must attribute the powers of ferments to definite atomistic or, speaking from an empirical standpoint, *materialistic* relations. This view receives support, above all, from the *specific nature* of the actions of ferments. Just as we base the chemical individuality of sulphuric acid on *specific reactions*, so, too, we are justified in the case of ferments in drawing the conclusion of specific material substrata from specific reactions. Whether, now, these are perfectly uniform, whether there is only *one* single chemical *diastase*, &c., or whether the starch-solvent action is a *group reagent*, and that there may be a whole series of diastases (which, by the way, is not improbable) is a matter of indifference for the argument.

That we have not here to deal with free wandering quantities of energy is shown *empirically* by the fact that, just as sound is only distributed in its substratum, the air, so, too, the actions of ferments never exceed the space limits of their material sphere.

Physiological considerations also support the view of the materiality of ferments, and, in particular, the fact that enzymes are *true secretions*, which are specially formed when the organism requires them (*vide infra*). It is very difficult to explain this fact in any other way than that physiologically required *substances* are here separated. And why does the *energy* of yeast invertase not pass into water until the yeast cell has been killed? All these questions can scarcely be answered from the point of view to which we are referring any more than the questions of *ferment immunity* and *anti-ferments*. The supporters of this view almost invariably draw a comparison with the magnet, the magnetism of which confers on it no specific material impress, but is only attached to it as a physical property.

Here, however, the case undoubtedly differs from that of the ferments. By suitable means this property can be taken from the magnet and again restored to it. But how can that be done with any ferment? There is no method of restoring its specific activity to a fermentative material which has once become inactive, when the ferment has actually been *destroyed*. Moreover, magnetism extends its influence only to intact molecules, but has not the power of forcing its way into the structure of individual molecules and causing disruption of the *atoms*. We must then, in considering the actions of ferments, always keep our mind fixed on the closely-related chemical energy. And as with the latter the specific action gives us the basis for a material individualisation, so, too, it is with ferments. We have every reason to assume that the specific activity is to be attributed to a specific material construction, to a particular grouping of the *atoms*. We shall deal more fully with this question later on.

We see, then, how confused is the whole question of the immateriality of ferments; theoretically it is untenable; but even considered from a purely empirical point of view it leads us not a step further. At the best it could only have the unfortunate result of causing strenuous attempts to isolate the material substrata of fermentative activities as chemical individuals to be abandoned.

We ought to avoid as far as possible, on practical grounds, building on such an insecure experimental foundation a theory

which cannot lead to anything as a *heuristic* principle, such as, in fact, the purely dynamic theory of ferments cannot be, but which, on the contrary, would always discourage us from new experimental investigations. This view is only supported by the fact that no ferment is yet known in a pure condition; it is therefore at present impossible to specify other characteristic properties in addition to their *actions*, and this is the reason why the substratum is lost sight of more than the activity. We must, however, hold fast to the notion that ferments are really chemical substances, whether of an albuminous or other nature. They are still, in every instance, unknown to us with certainty. We see ourselves compelled to leave the subject of "The Chemical Nature of Ferments" with still many notes of interrogation.

Of the other properties of ferments there is also little to be said. They are soluble in water and aqueous solutions of glycerin, and also in neutral solutions of salts, dilute alkalies, and acids.

They are precipitated, though not completely, by alcohol. According to DASTRE,¹ *trypsin* is soluble in alcohol of 40 per cent. strength; and *pancreas diastase* in that of 60 per cent. According to DE JAGER,² *saliva diastase* is soluble in alcohol (including absolute alcohol?), and *pepsin*, according to BARDET,³ has the same property.

They are, moreover, as a rule, simultaneously carried down when precipitates, such as, for example, of calcium phosphate or iron, are produced in their solutions. Certain colour reactions which they are said to give, such as, *e.g.*, the different colorations with *orcin* and *sulphuric acid* (WIESNER⁴ and others), as also their power of turning guaiacum tincture blue, are probably not reactions of the ferments themselves, but due to impurities.

In addition to this, we have, hitherto, only been able to study the influence of various physical and chemical agents on ferments with reference to the changes effected in their activity.

Dialysibility of Enzymes.—From the results of various investigations it appears that ferments only possess a slight degree of diffusibility.

¹ Dastre, *C.R. Soc. Biol.*, 1895, 414. *Arch. d. Phys.* [5], viii., 120, 1896 (Bibliography).

² de Jager, *Virch. Arch.*, cxi., 183, 1890.

³ Bardet, *Bull. d. la Soc. d. Therap.*, xviii., 13, 1887, quoted by Dastre, *loc. cit.*

⁴ Wiesner, *Sitzb. Wiener Acad.*, xcii., 1. (See also under *Diastase* and *Emulsin*.)

It varies in degree, however, with different individuals, and also depends on the kind of membrane used. According to FERMI and PERNOSI,¹ for example, pepsin passes through De la Rue's paper, but not through good parchment.

According to the same authorities the majority of ferments pass through a porcelain filter.

With regard to vegetable rennet, LEA² states that like the animal enzyme it is retained by a kaolin filter. CHODJUEW³ comes to the conclusion that although ferments are capable of dialysis, they dialyze exceedingly slowly. This property (which is best utilised with a porcelain filter) affords a means of separating enzymic activities from the vital forces of living cells, and is frequently used for that purpose, in addition to the more certain method of *poisoning* the cells (*vide infra*).

¹ Fermi and Pernossi, *Z. f. Hyg.*, xviii., 106, 1894.

² Lea, *Proceed. Roy. Soc. London*, xxxvi., 55, Nov., 1883.

³ Chodjujew, *Arch. d. Phys.*, 1898, 241 (Bibliography).

CHAPTER IV.

THE INFLUENCE OF EXTERNAL FACTORS ON THE
ACTIONS OF FERMENTS.

FERMENTS are affected in the most different ways by physical and chemical agents. Unorganised ferments differ considerably in their behaviour towards these influences; they are more or less resistant, the most sensitive towards all influences being Buchner's zymase (*vide infra*).

According to POZERSKI,¹ yeast, diastase, invertase, inulinase, pepsin, and trypsin can be cooled to -191° C. without injury.

Organised ferments are much more easily injured when associated with the protoplasm of their mother cell. DASTRE² has drawn up a scheme of these influences. He classifies them into four groups:—

1. *Zymoplastic momenta*³ are those which convert *zymogenes* (*proferments*) into active ferments; dilute acids, for instance, act as such.

2. *Zymo-stimulating* or *zymo-dynamic* (ARTHUS) agents are those which promote or accelerate the activity; *heat*, in particular, acts as such, as also dilute acids, and certain neutral salts; in some cases, too, dilute alkalies (*trypsin*) and carbon dioxide (*rennet*).

3. The *zymo-frénateurs* (ARTHUS).—These have an injurious effect on the action of ferments. When they entirely prevent the activity without destroying the ferments Arthus terms them *zyminhibiteurs*. Such are, notably, *cold* and *alkalies*, as well as all chemical reagents when they reach a certain concentration.

4. *Zymolysis*, which involves a complete disruption of the ferment. To this class belong, in particular, a high temperature of the solution, strong acids, &c.

Action of Physical Agents on Enzymes.—One of the most prominent properties of all enzymes, which, theoretically, is of

¹ *C. R. de la Soc. Biol.*, 1900, lii., 714.

² Dastre, *C. R. Soc. Biol.*, 1897, 469.

³ This designation is decidedly preferable to the "agent zymogénique" of Arthus (*Nature des Enzymes*, 1896, 13).

the greatest interest, is their sensitiveness towards high temperatures when they are in a state of solution. All enzymes exhibit an *optimum* of activity between 35° and 45° C.; at lower temperatures their activity rapidly diminishes, becoming insignificant at 0° C.; at higher temperatures a rapid *decomposition* of the ferment invariably sets in. Whilst dry ferments can be strongly heated, many of them far above 100° C., without their activity suffering (HUFNER,¹ SALKOWSKI²), they are, without exception, destroyed in an aqueous solution at about 70° C. In other solvents, notably in amyl alcohol, they are said to be more stable.³ According to PAVY,⁴ *pancreas diastase* and *liver diastase* can withstand boiling in absolute alcohol. The individual *death* temperatures of the different ferments vary within fairly narrow limits, and the statements on the exact points are therefore frequently at variance, the more so since this temperature is influenced to an extraordinary degree by the presence of *foreign substances*. The action is not a sudden one, but there is a *gradual weakening* which finally ends in "death." As a rule, the thermal death point is *higher* when the ferment is heated in admixture with its *substratum*, as, for example, *diastase* with *starch paste*, &c.

According to D'ARSONVAL,⁵ very low temperatures—down to -50° C.—have no action upon enzymes; at -100° C. invertase becomes inactive, but not yeast.

Sunlight, too, rapidly destroys enzymes in aqueous solutions, but not when they are in a dry condition or dissolved in indifferent liquids.³

The action of sunlight upon diastase has also been thoroughly investigated by GREEN⁶ (see *Diastase*).

Action of Acids and Bases.—On this subject we possess an immense amount of literature, which, in its main features, will be dealt with in discussing the individual ferments.

The only point which has been established beyond doubt is the *destruction of all ferments by mineral acids and alkalies when strongly concentrated*.

Moreover, it appears certain that *very dilute* acids stimulate all ferments to energetic activity. *Alkalies*, even when considerably diluted, only appear to be beneficial to *trypsin* and

¹ Hüfner, *J. pr. Ch.* (New Series), v., 372.

² Salkowski, *Virch. A.*, lxx., 158.

³ Fermi and Pernossi, *Ztschr. f. Hyg.*, xviii., 83, 1894.

⁴ Pavy, *Journ. of Physiol.*, xxii., 396, 1898.

⁵ D'Arsonval, *C. R. Soc. Biol.*, xlv., 808, 1892.

⁶ Green, *Philos. Transact.*, clxxxviii., 167, 1897.

similar ferments; the remainder are, for the most part, injured by them.

The *degree of the action* of individual acids and of different degrees of concentration on individual ferments is so variable, and the statements on the subject so contradictory, that it appears impossible to draw general conclusions from them. Generally speaking, organic acids appear to act less energetically than mineral acids. As regards *carbonic acid*, the statements are particularly at variance.¹

Action of Neutral Salts on Ferments.—Investigations on this point have been made in great number,² of which particulars will be found under the individual ferments.

Exceedingly little has resulted from all these isolated investigations, which are hardly capable of being summarised.

Salts appear to act much less in accordance with physico-chemical laws, depending in some way on the molecular concentration, than in accordance with specific chemical views. Only in this way can the great difference in the behaviour of different salts towards the same ferments, and of the same salt towards different ferments at equal degrees of concentration, be explained.

Moreover, the concentration of the substance undergoing fermentation appears to have an influence, to which conclusion we are led by the observations of, *e.g.*, KÜBEL,³ who found that the influence of common salt on saliva diastase varied with the concentration of the starch paste.

In general, dilute salt-solutions have a stimulating effect upon fermentative processes; above a certain degree of concentration, a retarding influence is exerted more and more, and finally a stage is reached at which the process comes to a standstill.

The influence of the *salts of heavy metals* has also been investigated, and in like manner found to be very variable.

Borax is stated by DUMAS⁴ to have a restrictive effect on all the ferments examined by him, but it is said not to hinder alcoholic fermentation (SCHÜTZENBERGER).⁵

Attempts have been made in various ways to discover the laws underlying this influence. An idea of NASSE's⁶ that salts acted injuriously upon ferments in proportion to their *dehy-*

¹ See especially Schierbeck, *Scand. A. f. Phys.*, iii., 344.

² The most comprehensive are those of Fermi and Pernossi, *Z. f. Hyg.*, xviii., 96, 1894.

³ Kübel, *Pflüg. A.*, lxxvi., 276.

⁴ Dumas, *Compt. Rend.*, lxxv., 295.

⁵ Schützenberger, *loc. cit.*, 246.

⁶ Nasse, *Pflüg. A.*, xi., 145, 1875.

drating power, which found its expression in a diminished vapour tension, was found by Nasse himself to be irreconcilable with the facts.

On the other hand, the notion was expressed in various directions that the ferments must enter into definite combination with the salts to form more or less stable compounds, but for this closely-allied view also no proofs were given.

Influence of Protoplasm-Poisons.—Whilst the vital activity of micro-organisms is crippled by many kinds of poison, the enzymes on the other hand are relatively insusceptible. It was thus a great advance when we learnt how to eliminate the activity of micro-organisms (especially of those of putrefaction) in order to distinguish the pure enzymic action in experiments on fermentation.

The principal substances used for this purpose are :—Alcohol, ether, ethereal oils (BOUCHARDAT¹), salicylic acid (KOLBE²), thymol (LEWIN³), chloroform (MÜNTZ,⁴ SALKOWSKI⁵), toluene (E. FISCHER⁶), sodium fluoride (TAPPEINER,⁷ ARTHUS and HUBER⁸), calomel (WASSILIEFF⁹), mercuric chloride¹⁰ and sodium azoimide (LOEW¹¹).

There are, however, numerous observations extant which show that these poisons do not leave the enzymes entirely uninfluenced,¹² but have an injurious effect, though not a very pronounced one, on their activity; this holds good, especially in the case of salicylic acid,¹³ phenol¹³ (PLUGGE¹⁴), thymol,¹⁵ and chloroform,¹⁶ as also for sodium fluoride (PAVY¹⁷), whilst toluene appears to have the least influence.

Alcohol, which was formerly extensively employed to exclude undesirable micro-organisms, usually also acts injuriously on

¹ Bouchardat, *Ann. d. Chim. et Phys.* (3), xiv., 61.

² Kolbe and his pupils, *J. pr. Ch.*, New Series, x., xi., xii.

³ Lewin, *C. med. Wiss.*, 1875, 324. ⁴ Müntz, *C.R.*, lxxx., 1250, 1875.

⁵ Salkowski, *D. med. Woch.*, 1888, 16.

⁶ E. Fischer and others, *Z. physiol. Ch.*, xxvi., 75, 1898.

⁷ Tappeiner, *A. f. exp. Path.*, xxvii., 108, 1890.

⁸ Arthus and Huber, *A. de physiol.* [5], iv., 651, 1892.

⁹ Wassilieff, *Z. physiol. Ch.*, vi., 112, 1882.

¹⁰ Mrotschkowsky, *Unorgan. Ferm.* Diss. Petersb., 1891, quoted by Kionka, *D. med. Woch.*, 1896, 612. Cf. Fermi, *Arch. f. Hyg.*, xii., 238.

¹¹ Loew, *Ber. d. d. chem. Gesells.*, xxiv., 2947, 1891.

¹² Treyer, *Arch. d. phys.*, 1898, 672.

¹³ Müller, *Journ. pr. Ch.*, x. (New Series), 444.

¹⁴ Plugge, *Pflüg. Arch.*, v., 549.

¹⁵ Among others Schützenberger, *Virch. A.*, cxxv., 340.

¹⁶ Among others Pugliese, *Pflüg. A.*, lxix., 115, 1898.

¹⁷ Pavy, *Journ. of Physiol.*, xxii., 391, 1898.

enzymes, apparently least so on pepsin (BUCHNER¹), but more energetically on *diastase* (WATSON²) and *invertase* (A. MAYER³). It destroys *maltase* very rapidly (E. FISCHER⁴), yet only in the presence of water (HILL⁵). *Hydrocyanic acid* interferes but little or not at all with the activity of ferments, but destroys their power of decomposing hydrogen peroxide (FIECHTER⁶), as is also the case with *cyanamide*, *acetonitrile*, and *hydroxylamine* (JACOBSON⁷). A simple current of air, however, is sufficient to expel the hydrocyanic acid and restore this power. A kind of loose combination appears to be formed here. We have already mentioned that formaldehyde renders ferments inactive and that LOEW infers the presence of amido-groups from this fact.

Alkaloids act in the most diverse manner, in some cases stimulating the activity and in others checking it (e.g., NASSE,⁸ SCHULTZ-SCHULTZENSTEIN⁹).

Tannin checks the action (SCHULTZ-SCHULTZENSTEIN⁹). *Phenol* is stated by ZAPOLSKI¹⁰ to have an injurious effect upon pepsin, but not upon diastase. DETMER,¹¹ however, proves that it also injures *diastase*.

NASSE and his pupils have studied the simultaneous influence of factors working in opposite directions upon ferments. They have found that there is a genuine *antagonism*: when an enzyme is subjected to the simultaneous influence of a stimulating and retarding medium, the final resulting action is equal to the arithmetical mean of both actions.

Similar experiments have been tried, e.g., by BAUM¹² on *invertase*. Potassium chloride acts to an appreciable extent as a stimulant, whilst ammonium chloride checks the action. Quinine and curare are in like manner antagonistic. The simultaneous influence of these substances on the ferments has the result described above. NASSE¹³ has made use of these experiments in investigating the influence of poisons and antidotes on *living cells*.

¹ Buchner, *Arch. f. klin. Med.*, xxix., 537.

² Watson, *Journ. Chem. Soc.*, xxxv., 539, 1879.

³ A. Mayer, *Enzymologie*, Heidelb., 1882, 13.

⁴ E. Fischer, *Z. physiol. Ch.*, xxvi., 74, 1898.

⁵ Hill, *Journ. Chem. Soc.*, lxxiii., 634, 1898.

⁶ Fiechter, *Wirk. d. Blaus. auf. Fermente*, Diss. Basle, 1875.

⁷ Jacobson, *Z. physiol. Ch.*, xvi., 367, 1892.

⁸ Nasse, *Pflüg. A.*, xi., 159, 1875.

⁹ Schultz-Schultzenstein, *Z. physiol. Ch.*, xviii., 131, 1894.

¹⁰ Zapolski, *Hoppe-Seyler's Medic. chem. Unters.*, iv.

¹¹ Detmer, *Z. physiol. Ch.*, vii., 1, 1882.

¹² Baum, *Antagonismus*, Inaug.-Diss. Rostock, 1892.

¹³ Nasse, *Maly's Jb.*, 1892, 584.

Action of the Decomposition-Products of Ferments on their Activity.—As a rule, ferments do not appear to be interfered with to any considerable extent by the decomposition-products, which they form in the course of their activity.

An exception to this rule must naturally be made in the case of very sensitive ferments intimately connected with the cells which produced them, which are destroyed by the *poisons* formed in their action. Thus the action of yeast upon glucose is finally crippled when a certain concentration of the alcohol produced is reached.

If the substances produced are *acids*, as is naturally the case in *acetic* and *lactic acid fermentations*, this interference with the activity of the enzyme is still more easy to understand, even when, as in the acetic fermentation, adaptation is the result of great habituation to the acids.

On the other hand, the *action of diastase*, for example, is not checked by the accumulation of sugar.¹

Peptones, on the contrary, appear to act as stimulants not only to the *proteolytic ferments*, but also to others²; this is opposed to the conclusion of KÜHNE, who has ascribed a restraining influence to the peptones.

TAMMAN,³ on the other hand, has proved that *emulsin* is extremely susceptible to injury from its own decomposition products; this being shown by the facts that not only was the activity of the ferment checked by the artificial addition of these substances, but also that it became more vigorous on the removal of the products naturally formed. When he added to a mixture of amygdalin and emulsin one of the decomposition-products, the decomposition was retarded, hydrocyanic acid being the most active in this respect, benzaldehyde less so, and glucose the least. But even the last-named substance had a more pronounced action than ether or alcohol. Unfortunately he made no experiments with substances of a similar nature to benzaldehyde, but not specific decomposition-products; it is quite possible that he would have found similar retarding influences in the case of, say, nitrobenzaldehyde or the like. Alcohol and ether were, however, in any case, not suitable objects of comparison. He obtained analogous results in the case of *salicin*. HILL⁴ found *glucose* to have a restrictive influence on the action of *maltase*:

¹ See also Wortmann, *Z. f. physiol. Ch.*, vi., 324. Cf. however under *Diastase*.

² Among others Chittenden and Ely, *Journ. of Physiol.*, iii., 327.

³ Tamman, *Z. f. physiol. Ch.*, xvi., 291, 1892.

⁴ Hill, *loc. cit.*

and invert sugar was found by MÜLLER-THURGAU¹ to interfere with the action of invertase.

Action of Other Chemical Agents.—Paul Bert² has stated that *compressed oxygen* injures the vital activity of micro-organisms without affecting enzymes.

Recently the influence of gases on ferments has again been examined; NASSE³ found oxygen and carbon monoxide to have an injurious effect on *invertase*. In the case of *hydrogen sulphide* FERMI and PERNOSSI⁴ were only able to establish an injurious effect on the gelatin-dissolving powers of certain bacteria, but not on the *enzymes* examined (*pepsin*, *trypsin*, *diastase*, and *emulsin*).

A remarkable fact, which has, however, been confirmed on many sides, is that normal blood-serum has a restrictive influence, more or less pronounced according to circumstances, on the activity of ferments (PUGLIESE and COGGI,⁵ HAHN,⁶ CAMUS and GLEY,⁷ RÖDEN.⁸

Reciprocal Influence of Ferments.—We have only scanty and, in part, contradictory information on this important question.

Only one fact is definitely established—viz., that *pepsin* renders *inactive* almost all the other unorganised ferments (*e.g.*, *diastase* and particularly *trypsin*), but that conversely *trypsin* has not the slightest influence on *pepsin*. On the other hand, *trypsin* destroys Buchner's zymase, which is also destroyed by the proteolytic ferments of *bacteria* and of *yeast itself*. *Pepsin*, again, by itself, has no action upon lactic acid fermentation. In all these cases it is difficult to take into account the deleterious action of the hydrochloric acid itself, without which the *pepsin* will not act, so that it is impossible to draw any valid conclusion as to the albuminous nature of enzymes from their decomposition by *pepsin*, even when this decomposition is not disputed, as in the case of *diastase*.

Behaviour of Ferments towards Hydrogen Peroxide.—As a result of the view that the actions of ferments were very closely allied to those of simple inorganic catalysing agents, the belief also arose that the property, common to all active solutions of ferments, of decomposing hydrogen peroxide in this way was a typical catalytic reaction of all ferments, and was to be regarded

¹ Müller-Thurgau, Thiel's *Landwirthsch. Jahrb.*, 1885, 795.

² Bert, *C.R.*, lxxx., 1579.

³ Nasse, *Pflüg. Arch.* xv., 471.

⁴ Fermi and Pernossi, *Zeitschr. f. Hyg.*, xviii., 92, 1894.

⁵ Pugliese and Coggi, *Maly's Jb.*, 1897, 832.

⁶ Hahn, *Berl. klin. Woch.*, 1897, 499.

⁷ Camus and Gley, *C.R. Soc. Biol.*, xlix., 825, 1897.

⁸ Röden, *Maly's Jb.*, xvii., 160, 1887.

as an integral part of their activity. Stress was laid upon this by SCHÖNBEIN¹ in particular; NASSE² then examined the influence of foreign substances on this property with the intention of investigating the action of the ferment itself in an indirect way.

This decomposition of hydrogen peroxide is recognised by the liberated oxygen causing an *alcoholic solution of guaiacum* to turn blue. We shall deal with this *guaiacum reaction* on several future occasions.

JACOBSON³ vigorously attacked the view which had been generally accepted up to that time, and was able to show that a complete parallelism between genuine fermentative activity and the decomposition of hydrogen peroxide in the above sense did not exist. On the contrary, he could effect a separation of catalytic force from fermentative force in three ways.

Emulsin lost its catalytic power completely at 72° C., and an *infusion of pancreas* at 62° C., whilst their fermentative powers remained intact, or at least partially so, at those temperatures.

Similar differences were observed on heating the dried ferments to 130° C. and 120° C. respectively. The catalytic force could be removed by an addition of hydrogen peroxide without the fermentative power being destroyed.

In like manner, "salting-out" the ferments with sodium sulphate proved a third means of causing the loss of the catalytic force without destroying the specific decomposing power.

Moreover, further differences appeared. Whereas, as is well known, small quantities of acids promote the fermentative activity, and alkalies interfere, the reverse is the case with the catalytic power; even insignificant amounts of acid retard and destroy it, whilst very dilute alkali at first *promotes* its action, although indeed in stronger concentration it, too, has a restrictive influence.

Salts, almost without exception, retard the liberation of oxygen, though to a very variable extent. In the case of *pancreas ferment* potassium sulphate in a 4 per cent. solution promotes the liberation of oxygen; the influence of other salts is weaker.

With sodium phosphate and some few other salts, an acceleration can be recognised. *Potassium sulphocyanide* has a very strong retarding influence.

Most characteristic of all is the action of *hydrocyanic acid*, which inhibits the decomposition of hydrogen peroxide altogether, or to a very great extent, without affecting the action

¹ Schönbein, *J. pr. Ch.*, lxxxix., 334. ² Nasse, *Pflüg. A.*, xi., 159, 1875.

³ Jacobson, *Z. f. physiol. Ch.*, xvi., 340, 1892.

of the ferment. A similar result is produced by *cyanamide*, *acetonitrile*, and *hydroxylamine*.

It follows from this that the decomposition of hydrogen peroxide is *not* a function inseparably associated with ferments, but can be separated from their fermentative activity. It is impossible to say whether foreign substances are here present, or whether the material substrata of the ferments themselves possess atomic groups, to which this capacity is attached.

Notwithstanding this, GRÜSS,¹ at the present time, has claimed the properties of "oxydases" for the diastases of higher plants—i.e., attributed the *guaiacum* reaction with hydrogen peroxide to the diastase itself.

Very interesting analogies have been drawn by BREDIG and MÜLLER VON BERNECK² between this special catalytic action of ferments and the decomposition of hydrogen peroxide effected by *colloidal platinum* in an aqueous or slightly acid solution. In this case, too, slight traces of platinum (1 gr.—atom in several million litres of water) act very energetically on much greater quantities of hydrogen peroxide.

Far-reaching analogies with the catalytic force of ferments have also been pointed out with regard to the influence of *temperature* on the velocity of the reaction, and the retarding effect of certain salts, &c. Particularly striking, however, is the fact that the specially injurious effect of *hydrocyanic acid*, as also of *hydrogen sulphide* and *mercuric chloride*, was also observed in the case of colloidal platinum; for even on the addition of hydrocyanic acid proportion of 3 : 1,000,000, the catalytic force decreased to one-half.

However, just as we have seen that the catalytic force can be separated from the specific fermentative actions, so, too, in the following chapter we shall have an opportunity of showing that in a corresponding manner the physico-chemical investigations of *fermentative actions* have led to the conclusion that they do not follow the laws of simple catalysis, but show characteristic differences. Thus, when Bredig and Müller describe their colloidal platinum as an inorganic ferment, the term is not entirely free from objection, apart from the fact that we must oppose it fundamentally, since, according to our definition, we describe ferments as the *products of living cells*. But even with reference to their action we must separate catalytic force, which, according to OSTWALD, is only an acceleration of simple chemical reactions, from the specific actions of ferments.

¹ Grüss, *Ber. d. botan. Ges.*, 1898.

² Bredig and Müller von Berneck, *Zeitschr. f. physikal. Ch.*, xxxi., 258, 1899. Quoted by Bredig in the *Nat. Rdsch.*, 1900.

CHAPTER V.

THE MODE OF ACTION OF FERMENTS.

As we have already briefly pointed out, we can divide the chemical actions of ferments into two main groups. The purely *hydrolytic decompositions* are brought about by a molecule of complicated structure breaking up into simpler decomposition-products, with an accompanying absorption of the elements of water. These decompositions take place in a manner *analogous* to those produced by hydrolysis by means of *acids* or *alkalies*, and HOPPE-SEYLER¹ has therefore classified the hydrolytic fermentative actions together.

The *hydrolytic fermentations* which are analogous to the decomposition effected by acids are as follows:—

A. *The Disruption of Carbohydrates.*

1. The decomposition of *starches* into dextrins and maltose by the *diastatic* ferments.

2. The decomposition of other higher carbohydrates which has not been so thoroughly investigated—of *cellulose* by *cytase*, of *inulin* by *inulase*, of *mannan* and *galactan* by *seminase*, of *carubin* by *carubinase*, and of *pectins* by *pectinase*.

3. The decomposition of *maltose* into two molecules of *d-glucose* by *maltase*.

4. The decomposition of *cane sugar* into one molecule of *d-glucose* and one molecule of *d-fructose* by *invertase*.

5. The decomposition of *trehalose*, *melibiose*, and *lactose* by ferments which have as yet been but little investigated—*trehalase*, *melibiase*, and *lactase*.

B. *The Decomposition of Glucosides* by special enzymes, in which one of the decomposition-products is *d-glucose*.

C. *The Decomposition of Urea into Ammonium Carbonate* by *Urase*.

D. *The Decomposition of Albuminous Substances.*

1. Through the action of *pepsin*, substances of lower, though still high, molecular weight are produced—*albumoses* and

¹ Hoppe-Seyler, *Pflüg. A.*, xii., 1.

peptones—in the same way as they are formed by the action of *dilute acids* on *proteids*.

2. *Trypsin* effects the decomposition in an analogous manner to concentrated acids: crystalloid, relatively simple bodies are produced, principally ammonia, amido acids, and di-amido acids.

3. *Rennet* brings about a decomposition of casein (which has as yet been insufficiently examined, but is probably also of a hydrolytic character), with the formation of a coagulated albuminous substance, paracasein, as is also possibly the case with *fibrin ferment*¹ and the coagulation of pectinous substances by *pectase*. In both the latter cases, however, it is still very doubtful whether there is any fermentative action. Analogous hydrolyses occur in the decompositions effected by alkalies.

E. *The Fat-Decomposing Enzymes* which transform glycerin esters into fatty acids and glycerin.

F. *The Lactic Acid Ferment* which produces lactic acid from sugar.

It is more difficult to follow the chemical action of *oxidising ferments*.

Here, also, two groups can be formed:—

I. *The Oxydases*, which derive the necessary oxygen for the oxidation of their substratum *from without*.

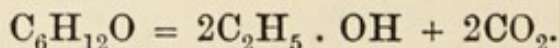
A. From the *atmosphere*, which is the case—

1. In the activity of *true oxydases*, which act as a carrier of oxygen, as, for example, in the *oxidation of salicylic aldehyde to salicylic acid*.

2. In the oxidation of ethyl alcohol by means of the *acetic fermentation*.

B. From the decomposition of *hydrogen peroxide*, the oxygen of which is used for the oxidation. This is the case with the so-called “indirect oxydases.”

II. Quite apart from all other enzymic processes stands the enzyme of *alcoholic fermentation*, which can hardly be classed with the oxidising ferments, as the process is essentially represented by the equation,



It is far more likely that we have here to deal with a process which is carried on without any addition of *external* oxygen, which is, to a certain extent, an *intramolecular oxidation*, in which, with an accompanying evolution of heat (*i.e.*, an *exothermic process*), a fresh equilibrium is produced in such a

¹ I have special reasons for not designating the fibrin ferment more exactly.

manner that a part of the carbon in the molecule is completely oxidised *at the expense of the other part*.

With this proviso, we can also regard *zymase* as an *oxydase*, although of an altogether special kind.

In this way, then, we arrive at a relatively simple scheme of fermentative actions. The chemical differences which have been established between simple *decompositions* and *fermentations*, with the object of basing a primary separation of enzymes from organised ferments upon them, do not appear to me, from a purely dynamic standpoint, to be sufficiently important to support this otherwise untenable proposition. It has, for instance, been brought forward as a specific process of alcoholic fermentation that, during its course, combinations between carbon and carbon, carbon and hydrogen, &c., must be broken up, whilst new combinations of carbon and oxygen are continually produced.

HOPPE-SEYLER,¹ in particular, has devoted special attention to these chemical decompositions, and in his usual keen manner has followed the migrations of the oxygen atom and investigated their conditions.

However, I still believe that the chief stress must be laid upon the question whether the process is *endothermic*—i.e., attended with an absorption of energy—or *exothermic*, liberating energy in its course; the proximate conditions of this transformation are naturally very important in themselves and interesting to investigate, but—whether the oxygen atom travels more or less, or whether greater or smaller transmutations take place at the same time—a fermentative process can only be of an *exothermic* nature, and within these limits we need not make any far-reaching primary distinction between more or less complicated decompositions. On the other hand, such oxygen migrations, in Hoppe-Seyler's sense, naturally occur also in processes of which the total result is endothermic, and which thus belong to *metabolism*, so that we cannot count them among the fermentative processes.

With reference, then, to the kind and manner of fermentative decomposition-processes, we have to conceive ferments to be forces, which are endowed with the capacity of imparting to an unstable atomic system a shock, which shatters it at one spot, and induces decomposition in such a manner that this decomposition spreads throughout the whole substratum until a new condition of more stable equilibrium has been established. It is not altogether irrelevant to point out (if we may avail ourselves

¹ Hoppe-Seyler, *Pflüg. A.*, xii., i.

of Naegeli's theory for the purpose) that atomic vibrations already existing may become so intensified by the energy imparted by synchronous vibrations in the molecules of the ferment, if we may regard it in such a crude mechanical fashion, that they exceed their normal rhythm, and thus bring about a disruption of the molecule. Still, at the same time, we must not lose sight of the fact that this "explanation" only affords a very convenient *mode of representing* the nature of the process which we designate by a convenient generic term as *catalytic* or *contact action*. Such catalytic processes include not only fermentations, but also numerous processes of an *inorganic* nature. For closely analogous reasons the actions of enzymes have frequently been placed on a par with those of *mineral contact-substances*, as for example the oxidising action of finely-divided platinum or the hydrolytic action of very dilute acids.

By explaining inorganic contact action it was hoped that a key to the understanding of fermentative processes would be found. HÜFNER,¹ for instance, has proposed a very ingenious theory of contact action.

Hüfner first forms a theory of the attracting forces between two di-atomic molecules of which each atom attracts not only the other atom of the molecule, but also each of the atoms of the other molecule, and assumes that so long as the intramolecular forces of attraction outweigh the attractions of the foreign atoms, the molecules remain intact. He then constructs similar theories for the case of three molecules exerting simultaneous influence on one another. Under such conditions a case is also possible in which the forces are so distributed that two of the three molecules are broken up whilst the third remains intact.

He next considers the case of the decomposition of hydrogen peroxide by platinum black.

The hydrogen and oxygen atoms of the first molecule vibrate within it around a certain system of equilibrium; now each of these atoms is attracted by the platinum, but the oxygen atom more strongly than the hydrogen atom; it is now conceivable that just at the time when an oxygen atom is furthest away from its hydrogen atom the attraction of the platinum becomes stronger than the cohesion of the molecule, so that the latter is broken up.

The reason why such processes induced by *catulysis* are not also retrogressive, and why many transformations cannot com-

¹ Hüfner, *J. pr. Ch.*, new series, x., 385, 1874; cf. Loew, *J. pr. Ch.*, xi., 372, 1875.

mence at all without the assistance of catalysing agents, he ascribes to the restriction of the activity of chemical affinities to the most intimate contact and the rapid decrease in chemical attraction which accompanies the removal.

Owing to the fact that the molecules of "catalysing agents" are not broken up, but only "distended" in these processes, they possess this unlimited operative capacity.

I cannot here go into the details of this very interesting work, which to a certain extent presents a geometrical conception of Naegeli's moleculo-physical theory, although at times it goes beyond it. Although HÜFNER has also brought the ferments within the compass of these observations, yet he has not based so direct a *theory of ferments* on them as did Naegeli, and besides has only applied them to the unorganised ferments.

Many other attempts have been made to explain the action of ferments.

LOEW,¹ in his assumption of free aldehydic groups in enzymes (*vide supra*), also refers to the hydrating action of aldehydes, and attempts to base a parallelism with fermentative action on this. He points to the fact that *cyanogen*, on contact with acetaldehyde, absorbs water, and is transformed into *oxamide*. This was first recorded by LIEBIG.² SCHMITT and GLUTZ,³ however, found that this reaction was also brought about under the influence of hydrochloric acid. It has not been more closely investigated; in fact, the great instability of cyanogen would present considerable difficulties in the way of a more thorough investigation of this question.

The modern theories of electrolytic dissociation have become so recondite that no attempt has been made to find the weak spot in the baffling enigma by their aid. Now, there appears to me to be no doubt but that in this direction lies the road by which finally the goal will be reached, if ever it is reached. The accurate investigation of physico-chemical processes, of the alterations in *conductivity*, and in certain cases, perhaps, also in the osmotic pressure and velocity of reaction, as also of ionisation in general, are undoubtedly necessary preliminary work for the establishment of a theory of ferments on an exact basis.

Yet, in this direction, hardly the first step has been taken. An extremely interesting fact, however, the discovery of which we owe to that indefatigable investigator of ferments, Nasse, deserves mentioning here as of primary importance.

¹ Loew, *Pflüg. A.*, xxvii., *loc. cit.* ² Liebig, in his *Ann.*, cxiii., 246.

³ Schmitt and Glutz, *Ber. d. d. chem. Ges.*, i., 66, 1867.

NASSE¹ started from the very ingenious idea that a ferment at the moment of its *specific* activity must possess active *free* ions, which, by their kinetic energy, induce the process.

He accordingly investigated the conductivity of mixtures of *fresh* ferment and water, and of *boiled* ferment and water, and observed no greater conductivity in the case of the "raw" ferment. When, however, he replaced the water (which can also be represented by a solution of a *non-specific* substratum, as, for instance, cane sugar with diastase) by a solution of the specific substratum of the ferment, as, for example, starch with diastase, the "raw" ferment showed a greater conductivity than that which had been destroyed by boiling. It thus appears that an actual *dissociation of the ferment takes place in the course of its specific activity*. Unfortunately, these highly important experiments have never been confirmed and extended.

The fact that fermentative decompositions are frequently very similar in their course to acid-decompositions was very misleading, since it supported the assumption that we have here to deal with actually analogous processes. There was also frequently an inclination towards the idea that the typical decomposition finally merged into a genuine acid-decomposition, and that ferments only had an influence in accelerating and intensifying this acid-decomposition.

They do this, of course, but it is not easy to base a theory on the fact. And there are also general reasons which lead us to the conclusion that there is an essential difference between inorganic contact actions and true fermentative actions.

In the first place, the analogy with acid-decompositions only extends to the simple *hydrolytic decompositions*; oxidations, and especially alcoholic fermentation, are altogether outside the bounds of this possible explanation.

Moreover, very deep-seated differences can be observed by the scientifically exact methods of physico-chemical research between the course of simple catalytic processes, as, for example, hydrolysis by dilute acids, and the mode of action of ferments.

These differences are seen in the quantitative limitations of the processes, in the velocity with which they take place, and the influence which the quantity of the ferment, the decomposition-products formed, and the other *physical* conditions, notably the *degree of concentration* and the *temperature*, exercise on their activity.

All these respective conditions have been thoroughly examined

¹ Nasse, *Maly's Jb.*, 1894, 718.

by G. TAMMAN¹ in a comprehensive research, and he has treated them so exhaustively that there appears scarcely any other course open to me in elucidating this difficult problem than to adhere, in the main, to his work.

Ferments act *hydrolytically*. We will for the present leave out of the consideration oxidising ferments, the activity of which has as yet been scarcely investigated from a physico-chemical point of view. Water by itself has a slight hydrolytic action. This hydrolysis is, in the first place, increased by heating. Thus, MUNK² succeeded in decomposing glucosides by heating them with water at 150° to 160° C. It is, further, strengthened by *acids*, although all acids are only effective according to the proportion of their *hydrogen ions*, which are alone to be taken into account (ARRHENIUS³). These are also present to a slight extent in not absolutely pure water, and cause a slight hydrolysis. But this does *not* explain the action of ferments, for they are not electrolytes, and thus have no free hydrogen ions. Ferments, therefore, exercise their power of accelerating hydrolysis *in a different manner* to acids. They have, as we shall subsequently show in detail, definite atomic groupings which enter into relation with certain groupings of the substratum. On this is based a primary, most important distinction, the *specific nature of ferments*.

There are, however, further differences. *The reactions of ferments are incomplete*. Tamman easily shows by reference to literature, and his own experiments, that the ferment never completely decomposes the substance on which it acts, but invariably leaves an undecomposed residue. The only exceptions are *rennet*, which always precipitates the *whole* of the casein, and apparently, at any rate at higher temperatures, *invertase*.

This incompleteness, however, is not perhaps the result of the establishment of a *condition of equilibrium*, as, for instance, happens in the decomposition of an *ester* by an acid, in which, in addition to the hydrolytic decomposition, there is a re-formation of a fresh ester. Fermentative action, on the contrary, only proceeds *in one direction* without any *synthetical retrograde formation*, as Tamman also illustrates experimentally, quite in accordance with our frequently expressed view, that ferments are *never* able to bring about synthetical endothermic processes.

That this process cannot be the realisation of a state of equi-

¹ Tamman, *Z. f. physiol. Ch.*, xvi., 271, 1892.

² Munk, *Z. physikal. Ch.*, i., 357, 1877.

³ Arrhenius, *Z. physikal. Ch.*, iv., 226, 1889.

librium in the above sense is shown by the fact that it can be set in motion again by raising the temperature, which is not the case in the saponification of acids, in which the formation of a state of equilibrium is independent of the temperature.

Besides, it has also been proved that the process does not come to a standstill on the *destruction of the ferment*.

In direct contradiction to all previous investigations, we have HILL's¹ results, which urgently require confirming or refuting. Hill, in fact, claims to have established the astounding fact that glucose is *partially re-converted into maltose* under the influence of *maltase*, when in concentrated solution, but that with a concentration of less than 4 per cent., this reversion of the process does not take place. Equally concentrated solutions of glucose and maltose are stated to be formed under the influence of maltase from the same equivalent proportions of glucose and maltose. It is advisable to accept with great scepticism these results, in which the fresh formation of maltose is inferred from the observed parallel increase in the optical rotation and decrease in the reducing power; if confirmed, it would, of course, involve a quite exceptional separate position for the action of maltase.

The decomposition of the ferment as a cause of the cessation commences, however, at *higher* temperatures. The higher the temperature rises above the optimum, the quicker the process comes to its final stage, and at a certain temperature the ferment becomes inactive so rapidly that nothing, or practically nothing, of the substratum is attacked. And this end-stage is then a definite one; the ferment is destroyed or, as Tamman assumes, split up, and *nothing* restores its activity.

The decomposition of the ferment, however, does not occur at lower temperatures, since it is possible to again induce the process by means of external factors. Hence, in this case, only a *state of inactivity* has ensued. This *inactive form* can be converted into the active form by the following means:—

1. By raising the temperature. When, for example, an amygdalin-emulsin mixture has proceeded to its final point at 5°C., it remains constant at 5°C. If now it be heated to 40°C., the decomposition of the amygdalin continues its progress and does not again come to a standstill until it reaches the point, which has previously been attained by a mixture commencing at 40°C. The end-point is thus, *ceteris paribus*, dependent upon the final temperature, and it is a matter of indifference whether this point be reached immediately or in several stages. An

¹ Hill, *Journ. of the Chem. Soc.*, lxxiii., 634, 1898.

exception to this rule must naturally be made when the ferment begins to undergo a slow decomposition at the temperatures applied, since less substratum will then be decomposed in an intermittent progress to the end-point than in a decomposition in one stage. This is the case, for instance, with *invertase*, which, even at 20°C., begins to decompose.

The *second* method is a dilution of the mixture from time to time.

The *third* is the addition of *fresh* substrata. When *salicin* is added to an amygdalin-emulsin mixture which has reached its last stage, the decomposition of the salicin begins after a perceptible pause. The salicin thus effects the *resuscitation* of the emulsin.

The *fourth* is the addition of *fresh* quantities of active ferment when the end-stage is reached. The process then continues to a new end-stage, which is again influenced by the ferment, and so on.

Whence comes this inactivity? The *decomposition-products* appear to be responsible. In fact, an *artificial* addition of the decomposition-products induces an *earlier* appearance of the end-points; on complete removal of the decomposition-products, the decomposition continues *indefinitely*. This also gives the key to the divergent behaviour of *rennet*. Since, here, the *separation* of the decomposition-product, insoluble paracasein, takes place naturally, the *rennet* can continue its action without hindrance until the conversion of the casein is complete. *The inactive form of ferments is thus caused by the decomposition-products and can persist only so long as they are present* (TAMMAN). Since, on the other hand, it has frequently been asserted that the decomposition-products raise the "thermal death-point," Tamman assumes that the inactive ferment is decomposed more slowly than the active form.¹

Up to this point I have merely followed Tamman's conclusions. Before proceeding with them, I must introduce a few words of criticism. In the first place, Tamman has only demonstrated the incompleteness of fermentations in the case of a very limited number of ferments.

In the case of the proteolytic ferments, *trypsin* in particular, he has produced no proof, for the arrest of peptic digestion mentioned by him, depends upon the use of *hydrochloric acid*, and not on the enzyme becoming inactive.² Moreover, the

¹ This is stated of the peptonising action of *pepsin*, e.g., by Biernacki (*Z. f. Biol.*, xxviii., 62). Tamman gives the statement without a reference.

² Gürber, *Verhand. d. Würzburg phys. med. Ges.*, 1895 (cf. under *Pepsin*).

behaviour of trypsin does not support his view. I can affirm from my own experience that it is possible to carry a tryptic decomposition of albuminous bodies so far that only a minute quantity of a residue, probably consisting of impurities, is left, and that the solution contains absolutely *no* genuine albuminous substances, or even any *albumoses*, so that here we are justified in assuming a *continuous* decomposition of the protein, as, indeed, has also been affirmed on other sides (*e.g.*, by KUTSCHER). Of course, we might assume that the genuine fermentative decomposition-products are possibly different, but that they undergo a further change, either spontaneously or under the influence of the slightly alkaline medium, so as to lose their influence on the ferments, as Tamman points out is the case with *allyl mustard oil*, which changes into allyl cyanide (in the *decomposition by myrosin*); yet we should have no proof for this view. In any case, however, the matter is not so simple as this. And as regards the influence of the decomposition-products, *invertase* would form an exception, since there is not the same apparent reason for the inactivity of the decomposition-products in this case as with *rennet*. And it is conceivable that this ferment (*invertase*), so sensitive towards an elevation of temperature, when kept at *exactly* 40° C. (*i.e.*, a relatively high temperature) fulfils its task so energetically that it is not rendered "inactive." Moreover, we must take into account the fact that KÜHNE'S¹ view of the restrictive influence of peptones on pepsin, which Tamman quotes, has been directly contradicted —*e.g.*, by CHITTENDEN and ELY²; that in the case of *diastase* WORTMANN³ was unable to detect any injurious influence from the presence of even considerable quantities of sugar; and that in general the question whether the decomposition-products by themselves exercise an injurious influence on the action of the ferment is answered in the most opposite manner by authorities, even in the case of one and the same process. These remarks must be understood as only pointing out that the circumstances are not as simple as Tamman assumes, but not as depreciating the theoretical importance of his ingenious investigations. We absolutely agree with the conclusion that the activity of ferments cannot, without further proof, be identified with that of simple catalytic processes, but that they possess specific functions, the cause of which is for the present still veiled in darkness.

¹ Kühne, *Lehrb. d. phys. Ch.*, 1866, p. 39. Quoted by Tamman, *loc. cit.*, 294.

² Chittenden and Ely, *Journ. of Phys.*, iii., 327.

³ Wortmann. *Z. physiol. Ch.*, vi., 324, 1882.

The extremely interesting fact established by Tamman, that *emulsin* which has arrived at the end-stage of its process with *amygdalin*—i.e., at inactivity—still acts upon *salicin*, induces us to think that surely static conditions of equilibrium are here produced, although of a different kind to those in *saponification reactions*, and that the cause of these is a sort of combination, as it were, of the ferment with its substratum—a combination which precedes the decomposition. I should like subsequently to devote some attention to the subject of the possible nature of this combination. The fact before us is that in an *amygdalin-emulsin* mixture, a static condition can be established, which can be destroyed by external factors, such as heat, as also by the addition of fresh active material, either ferment or *another* substance, and is then transformed into dynamic actions. As to whether this amygdalin mixture could not also be again rendered active by a fresh addition of *amygdalin* no experiments have been made, although they would be very interesting from a theoretical point of view. Such a renewal of activity by the *same* substratum (*starch*) has been observed, for instance, by MORITZ and GLENDINNING¹ in the case of malt diastase (*vide* under *Diastase*.)

Significance of the Amount of Ferment for the Decomposition Process.—There are manifold reasons for concluding that ferments do not act like *the spark in a barrel of gunpowder*, so that an inappreciable emission of energy from them is sufficient to cause the process when once started to continue spontaneously to infinity, just as the total supply of coal could be consumed by the application of small flame. Although the amount of substratum which can be transformed is enormous as compared with the quantity of the ferment, so that, for example, one part of rennet, which is certainly, as yet, not pure, can decompose at least 400,000 parts of casein (HAMMARSTÉN), whilst invertase can transform 100,000 times its quantity of cane sugar (O'SULLIVAN and TOMPSON), and other enzymes similar colossal amounts; yet, however, it has *finite* limitations. Both the amount of the substratum decomposed in the final condition and the time occupied by the reaction are dependent upon the quantity of ferment added. But this only holds good up to a certain degree, for when we reach the ratio between the ferment and substratum at which the maximum of action occurs, the addition of *fresh* quantities of ferment is without influence. This is naturally also the case with temperatures at which no decomposition of the ferment sets in. Moreover, this phenomenon,

¹ Moritz and Glendinning, *Journ. Chem. Soc.*, lxi., 689, 1892.

which points to a quantitative relationship between ferment and substratum, lends support to the notion of a combination of the ferments with the substratum preceding the decomposition.

In addition to the quantity of ferment, the *degree of dilution* plays an important part in the formation of the final condition (although there are many exceptions, as, for instance, in the case of *salicin*), and the same may be said of the amount of substratum present, and, as we have already mentioned, of the *temperature*. The maximum of the final condition is thus influenced by *all* these factors. Tamman has attempted to investigate the counterbalancing influences, and proposes as a *probable* rule for a solution of *emulsin* and *salicin*, that "with an increasing amount of ferment, and a constant amount of *salicin*, the quantity of *salicin* decomposed at the temperature of the maximum increases in *arithmetical proportion*, whilst that of *emulsin* increases in *geometrical proportion*.¹"

At temperatures *below* the maximum the addition of an excess of ferment has no effect; at temperatures above the maximum the proportion of *salicin* decomposed still increases *with the quantity added*, though to a lesser extent than in the case given in the rule above, whilst naturally the total decomposition becomes less, and above the decomposition temperature of the ferment ceases. With small quantities of ferments the maximum ranges from 30° to 46° C. From the direction of the curves plotted by Tamman, we can draw conclusions as to *lower* temperatures (below 0° C.), and these indicate a similar "thermal death point" for such low temperatures. (According to Tamman it is -65° C. in the case of *salicin-emulsin*.)

Thus far Tamman's results. A striking point in them is the remarkably insignificant quantitative action of *emulsin*. He asserts that 0.01 milligramme of *emulsin* mixed with 0.25 milligramme of amygdalin effects so little decomposition that no hydrocyanic acid, and only the odour of benzaldehyde, can be detected. Other ferments, however, appear to act far more energetically. It must, therefore, be pointed out that Tamman's conclusions can, for the present, only be accepted for *emulsin*.

Velocity of the Reactions of Ferments.—Ferments are also distinguished from liberations of force of an explosive character by the *slowness* of their reactions. The rapidity of the reaction of ferments is influenced in the same way as the final stage by the *amount* of ferment and substratum as well as by the *temperature*. It is also affected by the presence of foreign bodies.

¹ Tamman, *loc. cit.*, 306.

The influence of the amount of ferment on the rapidity of the reaction has been investigated by, among others, BRÜCKE in the case of *pepsin*, by COHNHEIM for the *diastase of saliva*, by SCHWARZER for *malt diastase*, by BARTH for *invertase*, by MARKWORT and HÜFNER for *emulsin*, and by MAYER for *rennet*, &c. All these experiments have led to the conclusion that the time of the reaction decreases with the increase in the quantity of the ferment, yet is not directly in inverse proportion to it. This holds good for *rennet*, but only up to a certain proportion of the ferment (PETERS). TAMMAN¹ investigated the action of *invertase* on cane sugar, and found the curve *totally different* from that corresponding to the *acid-decomposition*. In the action of *emulsin* a maximum of activity is obtained with a given amount of ferment; any addition beyond that amount has no influence on the velocity of the reaction.

The quantity of *substratum* present has a converse influence in cases in which the amount of ferment is the same. The *speed of commencement* is specially affected by this factor; subsequently its influence becomes very trifling.

Greater concentration has at first a restrictive, but subsequently an accelerating, influence. More decomposition, however, is effected in dilute than in concentrated solutions in an equal time.

Of special importance for the conception of the separate position of ferments is the fact established by Tamman that the accelerating influence of temperature on the speed of reaction does not follow the curve which represents the acceleration of the *hydrolytic decomposition effected by acids*.

Of his results with individual ferments, reference should be made to the fact that, in the case of *invertase*, the rapidity of the start is retarded by temperatures below 40° C., but not at 50° C. Temperatures above 50° C. cause an increase in the rapidity of commencement, which, however, decreases with the rise of temperature. The reason for these deviations is that the ferment is more readily decomposed at higher temperatures. In consequence of this, the acceleration by ferments has a *temperature maximum*, above which it again falls. With regard to the influence of foreign substances on the speed and intensity of fermentative reactions, numerous investigations have been made (*vide supra*). A generalisation of the results, however, is impossible, owing to the statements being contradictory. The view that there is a difference from simple hydrolytic processes is supported by the fact that there are substances—*e.g.*, neutral

¹ Tamman, *loc. cit.*, 312.

salts—which promote the reactions of ferments, whilst, on the other hand, all *acid-decompositions* are influenced in their speed by every addition (NASSE).¹

We see, therefore, that we have every reason for assuming that ferments possess characteristic activities. From the results of physico-chemical investigation, it follows that any identification of these activities with the simple decompositions effected by acids is out of the question.

And, above all, the *specific action of ferments* is opposed to such a view.

Whilst it is possible to decompose starch, cellulose, albuminous bodies, glucosides, &c., by means of dilute acids under approximately the same conditions, ferments, under the same external conditions, develop a marked *specific* action. The *diastase* which decomposes starch has no action whatever either on *albuminous substances*, on *amygdalin* and *salicin* (COHNHEIM,² NASSE³), or even on the much more nearly related *disaccharides*, maltose and cane sugar, or probably even on *cellulose*, which is so closely akin to starch. In a perfectly analogous manner, the proteolytic ferments are devoid of all influence on *carbohydrates*, *fat*, &c.

And not only do the primary substances attacked vary according to the nature of the ferments, but the extent of the process also differs. Since achroodextrin and maltose are not susceptible to the action of *diastase*, the diastatic decomposition of starch ends with the formation of these products, whereas starch can be broken down directly into *d*-glucose by means of dilute acids under suitable conditions.

We are thus arrested by the problem: How is the specific ferment in a position to interfere, by the emission of energy, with the mechanism of the atomic vibrations?

The first glimmer of light was thrown into this dark corner by the ingenious experiments of EMIL FISCHER,⁴ who was the first to systematically apply the stereo-chemical mode of view to the actions of ferments.

That, as a matter of fact, the living cell is perfectly capable of distinguishing stereo-chemical differences in the structure of the molecule had already been shown by the experiments of PASTEUR,⁵ which proved that mould-fungi could only cause dextro-rotatory tartaric acid to ferment; and by the equally

¹ Nasse, *Pflüg. Arch.*, xi., 147.

² Cohnheim, *Virch. A.*, xxviii., 241. ³ Nasse, *loc. cit.*, 157.

⁴ E. Fischer, *vide Z. f. physiol. Ch.*, xxvi., 71, 1898.

⁵ Pasteur, *Comptes Rend.*, li., 298, 1860.

well-established fact that the fermentability of sugars by yeast is dependent on their stereo-chemical configuration. In the first place, speaking generally, only sugars with *six* and *nine* carbon atoms are fermentable, and of these, again, only those which, in their genetic structure, belong to the *d*-series,¹ and even those are only partially fermentable, notwithstanding the fact that they are structurally identical; but they are *stereo-chemically* different, and that is sufficient to make them partially inaccessible to the fermentative activity of the yeast.

E. Fischer now transferred this mode of considering the subject to the *enzymes*. He prepared artificial derivatives of sugar which were stereo-chemically different but structurally identical (*e.g.*, *methyl glucosides*, *esters* of sugar with methyl alcohol). With these he established two important laws:—

In the first place, the enzymes investigated by him (the enzymes of yeast infusion and emulsion) acted in general only upon the artificial glucosides of *fermentable sugars*, so that, by this fact alone, the clearest light was thrown upon the *essential significance of the stereo-chemical configuration*.

But, secondly, he obtained from fermentable sugars, also, *two stereo-isomeric series of glucosides*, which he termed α - and β -*glucosides*, and now the remarkable fact appeared that the glucosides of the α -series were *only* attacked by the enzymes of yeast infusion, and those of the β -series *only* by *emulsin*, so that by these results the specific action of ferments is apparently brought to a focus. In the special part we shall go more fully into these facts in dealing with the enzymes which decompose glucosides. And yet, on the other hand, in these results of Fischer there is, at the same time, also a *limitation* of the specific nature of enzymic activity. That enzymes need not work specifically, in the sense of only and exclusively directing their activity towards *one* chemical substance, is at once manifest, when we consider that *proteolytic enzymes* attack *all* albuminous substances, which are, however, undoubtedly different; that *diastase* decomposes *all* the varieties of *starch* (the different nature of which has not been proved) and part of the dextrins, and that *emulsin* effects the decomposition of numerous *glucosides*. And so Fischer was perfectly justified in assuming that the enzymes which decomposed artificially-prepared glucosides were identical with those which had long been known to decompose *cane sugar* and *maltose* on the one hand, and *amygdalin* and other *glucosides* on the other. It would be too improbable a

¹ The *actual rotation* is here irrelevant; the laevorotatory fructose, which systematically belongs to the *d*-series, is equally fermentable.

supposition to assume that *yeast-infusion* and *emulsin* contain enzymes exclusively adapted to artificially-prepared glucosides, which are never met with in nature.

We are thus gradually forced to the conclusion that there are definite *stereo-chemical atomic groupings* which can serve the ferments as a stepping-stone for their attack; that ferments can also be in a position to decompose several bodies of different *structure*; and that, provided they find *the atomic grouping suitable to them*, the structure may otherwise be what it will. And, if we follow this conception further, the analogy with the *toxines* of bacteria and the *vegetable toxalbumins* allied to them forces itself upon our notice in a shape which, although quite obscure and scientifically untenable, is irresistibly attractive.

The reader will, therefore, pardon me if I venture to follow up this conception.

The analogies between the *nature* and *actions* of ferments and those of *toxines* are unmistakable, and have frequently been dwelt upon for a long time past by ROUX and YERSIN, and now, in particular, in the case of *tetanotoxine*.¹

Both are bodies of high molecular weight and of unknown nature: apparently of an albuminous character, though losing more and more of their albuminous character with each advance in our knowledge of how to prepare them in a purer state.

Both are extraordinarily sensitive to acids and many other chemical agents; the activity of both is irrevocably destroyed by boiling.

Both are products of living cells. And, if we add to this that both develop their activity in such infinitely small quantities² that we cannot conceive of their action as a purely chemical or mass action, we have, indeed, analogies enough.

A further comparison may be made between the specific solvent action on hæmoglobin of *tetanolysine* (EHRlich) and the perfectly analogous action of ferments (HILDEBRANDT).³

It is also extremely enticing to attempt to explain fermentative action in the method which EHRlich⁴ has followed with such important results in the case of *toxines*.

¹ Vide Tizzoni and Cattani, *Arch. Ital. d. Biol.*, xiv., 105. On the other hand, however, the similarity of tetanus-poison and enzymes is very strongly disputed by Fermi and Pernossi (*Z. f. Hyg.*, xvi., 385).

² According to Brieger and Cohn, the lethal dose of tetanotoxine for a man was 0.00023 gramme in the case of a not perfectly pure poison! (*Z. f. Hyg.*, xv., 1, 1893).

³ Hildebrandt, *Virch. A.*, cxxi., 29, 1890.

⁴ Ehrlich, "The Oxygen Requirement of the Organism," Berlin, 1885; *Klin. Jahrb.*, vi. Cf. also Oppenheimer, *Biol. Centralbl.*, 1899, 799.

Ehrlich's view of the action of toxins is also a *stereo-chemical* one. According to his side-chain theory, the possibility of a toxin action depends upon the *haptophore* group of the toxin finding a corresponding *haptophore* group in the protoplasm of the cell to which it attaches both itself and the total molecule of the toxin. Not till then is the toxin in a condition to cause its *toxophore* groups to exercise their injurious activity on the cell. If the corresponding *haptophore* group is absent, the toxin has no power over the cell; this accounts for the *specific nature* of toxin activity.

In an analogous manner to Emil Fischer in his now celebrated simile of the *key* ferment which must fit the *lock* substratum, Ehrlich represents the combination of the toxin to the cell protoplasm.

If we could imagine that, in some similar way, the ferments possessed *haptophore* groups which corresponded to those stereo-chemically arranged groups of the substratum, and attached themselves to them; if, further, we imagined that, corresponding to the *toxophore* atomic grouping which caused the *physiological* dissolution, the death of the cell, there were a *zymophore* group which effected the *chemical* decomposition of the molecular grouping, we should have a concrete conception of the reason for the *specific activity* of ferments. Just as the *toxophore* group need not in itself be specific, but can be explained by the aid of chemical affinity as a perfectly simple physiological activity, so, too, the *zymophore* group which effected the decomposition need *not* possess *any specific property*, but might act in a manner comparable to that of simple *catalytic substances*, as, for example, simple acid-decomposition.

As soon as the ferment had attached itself to the substratum by means of the specific *haptophore* groups, the specific part of its activity would be attained, and the simple chemical action begun. And, just as in the case of simple crystalloid protoplasm-poisons, the physiological decomposition is not dependent on a specific stereo-chemical configuration—on *haptophore* groups—so, too, *simple acids do not act specifically in the hydrolysis*, but on all decomposable substances without selection.

We see how extremely attractive it is to follow this path; yet, at the same time, we must not lose sight of the fact that, notwithstanding all this apparent agreement, the difficulty of formulating a theory of ferments of this kind is enormous. We should have to take the tremendous step of drawing inductive conclusions from the protoplasm of the cell as to the configuration of such simple substances as *cane sugar* and *amyl-*

dalín, in order to assume the presence of similar haptophore atomic groupings in them; from the *physiological* action of the *toxophore* groups we should have to draw inferences as to the *chemical* action of the *zymophore* group; in short, such a theory can only be regarded, at any rate for the present, as a tentative experiment, which satisfies the mental craving for causation and analogy.

Yet there are already a string of facts which support such a view, apart from the numerous points of relationship in the nature of toxines and ferments, which we have described above. In the first place, an actual *combination of the ferment with its substratum* preceding the action, of the ferment, was recorded long ago. Fresh fibrin in particular has the power of combining with relatively large quantities of *pepsin*, *papain*, and *trypsin*, as well as of *diastase*, to form compounds so stable that they cannot be dissociated by washing.¹ A similar phenomenon has been recorded in the case of other ferments. This might be regarded as a *saturation of the haptophore groups on each side*.

This view is also supported by the fact established in the case of nearly all enzymes that the thermal "death" point is lower for *aqueous* solutions than for a *mixture of ferment and substratum*, so that the possible resulting "combination" of both appears to be more stable. Tamman's mode of expressing this is that the "inactive" ferment is more stable than the "active;" it is difficult to know, however, exactly what to make of that.

If, further, we wish to refer the action of the *zymophore* groups to a simple acid-decomposition, the actually observed combination of weak hydrochloric acid with the ferment, may, in turn, be brought forward in support of this view (see *Pepsin*).

The first unfavourable fact, which apparently cannot be reconciled with this attempt at explanation—viz., that ferments sometimes also combine with substances which they do not attack (*e.g.*, *pepsin* with *silk*), might be brought within the bounds of the conception by the assumption that although corresponding *haptophore* groups are present, they are not actually *zymophore*, so that notwithstanding a preliminary combination, no reaction takes place.

Of far more weight are the conclusions which can be drawn from the phenomena of *bacteriolysis* and *hæmolysis* (see *Special Part*).

Here without doubt we have to deal with true fermentative actions. When an *object injurious to the protoplasm*, such as a bacterium or a foreign blood corpuscle, has forced its way into the organism it is destroyed by the proteolytic ferment of the

¹ Szumowski, Bibliography, *Arch. d. Phys.*, 1898, 160.

blood. But in order to strengthen the proteolytic capacity of the blood, which is too weak by itself, an *entirely specific* ferment is produced by the stimulus of this injurious body¹ to act against it, and this, in the theory, attaches its haptophore group to that of the stranger, and *destroys* it with its *zymophore group*.

For this *one* special case we should in fact have the conception which is embodied in the theory of ferments as formulated above. Two *haptophore groups* coinciding with one another ("lock and key") and a subsequently active *zymophore group*. But unfortunately we cannot, as yet, generalise from this case.

Another important fact, which throws light from a new direction upon the analogies between toxines and ferments, has recently been discovered. One result of Ehrlich's side-chain theory was the assumption of the formation of specific *anti-substances* from the excess of haptophore groups produced, and that these circulating in the blood, attached to their haptophore groups intruding toxines, and rendered them innocuous²; the serum containing such anti-substances would then be capable of neutralising toxines in a test-tube. This is exactly what MORGENROTH³ has succeeded in showing in the case of rennet. In the same way as it is possible to stimulate the organism to produce anti-toxines by the gradually increasing introduction of toxines, he was able by means of increasing doses of rennet, to cause an "anti-lab" to be formed in the serum and milk of the animals upon which he experimented, which was capable of paralysing to a very great extent the action of rennet on the milk (*cf. Rennet*).

In this case, too, we have a complete analogy with the toxines. A substance provided with a haptophore group, formed from an excess of side chains, attaches the rennet to itself by means of its haptophore group, and so prevents it from bringing its *zymophore group* to act upon the casein.

¹ The "immune body" + the "addiment."

² *Cf.* my note in *Biol. Centralb.*, 1899, 799.

³ Morgenroth, *Centralbl. f. Bakt.*, xxvi., 1899.

CHAPTER VI.

THE PHYSIOLOGICAL ACTION OF FERMENTS.

It was naturally to be expected that ferments possessed of great activity should also not be without influence upon the living organism.

BÉCHAMP and BALTUS¹ found that subcutaneous injection of vegetable and pancreatic diastase had a poisonous action.

BERGMANN and ANGERER² proved that solutions of pancreatin and pepsin on injection raised the temperature, and were very poisonous. A similar result was obtained by ROUSSY³ in the case of a substance with inverting properties, which he isolated from decomposed beer, and to which he gave the very indefinite name of *pyréto-genin*.

MENDELSON⁴ found that pepsin caused fever in dogs, whilst the pressure of the blood was considerably increased.

HILDEBRANDT⁵ has examined more closely the physiological action of *pepsin*, *rennet*, *invertase*, *diastase*, *emulsin*, and *myrosin*.

All had a toxic action. The smallest lethal dose for a medium-sized rabbit was about 0.1 gramme in the case of *pepsin*, *invertase*, and *diastase*, and 0.05 gramme (or even 0.025 gramme after the lapse of a week and a half) with *emulsin* and *myrosin*. *Rennet* was much less poisonous; the lethal dose was 2 grammes.

Moreover, all caused febrile symptoms (up to 41° C.) when injected in a sterile solution containing 0.6 per cent. of ordinary salt, though more rapidly on intravenous than on subcutaneous injection. At first only the production of heat was augmented, but afterwards the liberation of heat as well, and this, on the decrease of the fever, showed a relatively greater increase than the production. Hildebrandt, on closer investigation, came to the conclusion that in these phenomena he was dealing with

¹ Béchamp and Baltus, *C.R.*, xc., 373, 539, 1880.

² Bergmann and Angerer, *Festschr. zum 500 j. Best. d. Univ. Würzburg*, 1882, 137.

³ Roussy, *D. med. Woch.*, 1889, 874. ⁴ Mendelson, *Virch. A.*, c., 291.

⁵ Hildebrandt, *Virch. A.*, cxxi., 1, 1890.

true febrile symptoms (*ferment fever*), inasmuch as antipyretics (kairin) were found to be effective.

The symptoms of the poisoning were:—Distaste for food, thirst, shivering, restlessness, staggering gait, and, finally, coma (in dogs). In the case of rabbits the main symptoms to be observed were emaciation, weakness, and, frequently, convulsions.

On dissection hæmorrhage of the internal organs was often found, and also fatty degeneration of the heart and liver, congestion of the kidneys, and thrombosis.

Rennet had only a very slight action, owing to the fact that the temperature of the body (about 40° C.) is no doubt injurious to it (MAYER¹). In the case of invertase, too, Hildebrandt succeeded in partially protecting the animals by artificial superheating, and hence he is inclined to regard the elevation of temperature in fever as, in general, a healthy reaction against the ferment.

Ferments were also found to be blood-poisons, bleaching and reducing the red blood-corpuscles. Whilst ferments *intra vitam* brought about coagulation of the blood, Hildebrandt confirmed the results of ALBERTONI² and of SALVIOLI,³ who found that when added to the blood outside the body they tended to prevent coagulation. On the addition of *fibrin ferment*, however, coagulation took place.

FERMI⁴ was inclined to attribute the fever-producing action and the accompanying symptoms of poisoning, following the injection of ferments, to the simultaneous introduction of pathogenic micro-organisms. Hildebrandt, in fact, had omitted to furnish convincing proof that his method of sterilisation really effected its purpose. This, however, was done by KIONKA,⁵ who confirmed Hildebrandt's results, using ferments proved to be sterilised (by means of *mercuric chloride* and a porcelain filter), and showed that the method of sterilisation adopted by Fermi (*loc. cit.*) had so weakened the enzymic activity that the ferments had no longer a toxic action.

FERMI,⁶ however, stoutly maintains his opposite view, so that uniformity on this point has not yet been obtained.

It is, nevertheless, highly probable that ferments actually possess properties which are also common to many bacterial

¹ A. Mayer, *Enzymologie*, Heidelberg, 1882.

² Albertoni, *C. med. Wiss.*, 1878, No. 36.

³ Salvioli, *C. med. Wiss.*, 1885, 913.

⁴ Fermi, *Arch. f. Hyg.*, xii., 238; xvi., 385, 1894.

⁵ Kionka, *D. med. Woch.*, 1896, 612.

⁶ Fermi, *Maly's Jb.*, 1897, 828.

toxines. HILDEBRANDT,¹ in a later research, was able to prove that ferments acted by *chemical contact*—i.e., attracted leucocytes—and thus produced local inflammations (e.g., at the point of injection).

He further investigated the avenues for the introduction of ferment "intoxication." In the stomach ferments were destroyed by the *pepsin*, but, on the other hand, clysters and dropping into the conjunctival sac proved effective.

Ferments proved to be capable of arresting the action of the newly-removed heart of a frog, and, in general, showed the characteristics of true protoplasm poisons.

Moreover, Hildebrandt succeeded in producing a certain degree of resistance to the poison in the animals on which he experimented, specially in the case of emulsin, and this resistance resembled a true immunisation in the facts that the animals not only readily withstood greater doses of emulsin, but also that the specific activity of the ferments in the organism appeared to be at least considerably weakened.

In addition to these ferment-immunisations, mention should also be made of the attempts to produce, by the introduction of diastase, immunity against the saccharifying ferment, the excessive activity of which is said to contribute to the development of diabetes. In these experiments KUSSMAUL² asserts that he has observed a diminution in the excretion of sugar after the intravenous injection of diastase, and the same result has been obtained by LÉPINE.³

¹ Hildebrandt, *Virch. Arch.*, cxxxi., 5, 1893.

² Kussmaul, *A. f. klin. Med.*, xiv., 1874.

³ Lépine and Barral, *C. R.*, cxiii., 1014, 1891.

CHAPTER VII.

THE SECRETION OF ENZYMES.

ALTHOUGH, for the reasons given above, we must altogether refuse to accept the purely biological view of fermentations, yet the practical significance of these processes for biological problems is so important that it behoves us to deal with them more fully.

We regard ferments as true *secretion products* of the living protoplasm, which, however, are *in part* distinguished from ordinary secretions by the fact that they remain in more or less intimate connection with the living cell, so that their *zymophore group*, if I may venture to use the expression without encroaching, still remains in combination with the germ of the protoplasm molecule. This connection has hitherto been either altogether inseparable; so that *these* ferments form, to a certain extent, the residue of the *organised* ferments in the older sense. Or, again, this bond may be broken by forcible means, which destroy or weaken the cell as a living individual—the group of enzymes thus obtained standing midway between the “organised” and “unorganised” ferments; some of these are also met with under other conditions as free excreted ferments. One, however, which can only be isolated by extraordinarily energetic action on the cell—Buchner’s *zymase*—will, *in practice*, long be designated as an organised ferment, whilst the others, particularly *maltase* and *invertase*, will, *in practice*, be assigned to the enzymes.

We have thus to differentiate these groups of ferments, viz., those firmly attached, those attached but *separable* from the vital process, and the simply *excreted* ferments.

The first group comprise—

1. The *lactic acid fermentation*.
2. The *acetic acid fermentation* and some other oxidising fermentations.

The second group consists of—

A. *Enzymes of the Disaccharides.*

1. Invertase.
2. Maltase.
3. Lactase.
4. Trehalase, &c.

B. The *Urea-decomposing Enzyme*—*Urase*.

C. Buchner's *Zymase*, and some other enzymes as yet not closely investigated, which are isolated in the same way.

The remaining enzymes are given up without difficulty to surrounding media; thus they can be detected in aqueous and glycerin extracts, &c. They form the *third group*. Although nothing can be stated as to the conditions of secretion of the enzymes of the first group, since they are not known outside the cell and the conditions of their activity are bound up with those of their parent cells, much research has been made on the mode of secretion of the second group of enzymes. The study of the enzymes of *yeast* has been of special importance in this connection. The living healthy yeast cell usually yields to an *aqueous infusion*, only a trifling amount of one ferment—*yeast diastase*. If, however, the yeast be killed or weakened by chemical or physical means, the complexion of affairs is essentially changed.

The following methods are used for this purpose:—The vital energy of the yeast is either weakened by letting it dry in the air at a temperature not exceeding about 30° C. When it is dry it can be weakened still more by heating it for a short time at 100° C.

Or another method is the application of protoplasm-poisons, which destroy the characteristic alcoholic fermentative capacity without injuring the other enzymes; toluene, chloroform, ether, salicylic acid, and thymol are used for this purpose.

There appears, however, to be a partial difference here, for although the enzymic action can be *recognised* in *poisoned yeast*, the enzyme does *not* pass into the infusion so long as the *cell-wall* is uninjured; at least, this has been established in the case of certain yeasts. It has thus hitherto been impossible to definitely decide whether the enzymes are in some way or other combined with the protoplasm, or are merely *retained* by the intact cell-wall.

Yeasts treated in this way have the following characteristics:—

Ordinary yeasts, which are capable of fermenting cane sugar and starch, yield in addition to the starch-decomposing *diastase* two further enzymes—*invertase*, which decomposes cane sugar,

and *maltase*, which decomposes maltose—and also in most cases *trehalase*, which has as yet been but little studied.

Certain yeasts are lacking in one or other of these enzymes; thus, *Saccharomyces Marxianus* contains only *invertase*, *S. octosporus* only *maltase*, *S. apiculatus* neither, &c. The *bottom fermentation* yeasts produce also a special (?) ferment, *melibiase*.

Under the same conditions, the *milk-sugar yeasts* yield, instead of *maltase*, an enzyme, *lactase*, which decomposes lactose; this, however, only passes to a very slight extent into an aqueous infusion.

Especially interesting is the case of *Monilia candida*. This mould-yeast contains a ferment which *inverts* cane sugar, since it is capable of causing that sugar to ferment. This *invertase* of *Monilia* cannot, however, be *isolated* from the cells by any method. But that, even in this case, there exists a ferment which is independent of the vital process is manifest from the fact that the yeast still *inverts* cane sugar after it has been deprived of its *fermentative power* by *toluene*, &c. We must then either assume with FISCHER¹ that *Monilia invertase* is insoluble in water, or that the cell-wall of this yeast is capable of such resistance that even after drying and heating it remains *impermeable* to the enzymes. What was stated above of *lactase*, which only passes in *slight* amount into the infusion, thus holds good in the widest sense of *Monilia invertase*.

This attachment of *invertase* and *maltase* to the cell is only met with, however, in the case of the enzymes of the yeasts and certain mould fungi. They also occur in another form as *free excreted* enzymes, especially in the sap of plants, as we also know to be the case with pancreas-maltase, intestinal *invertase*, &c.

Another enzyme which does not leave the healthy cell is the ammonia-producing *urase* of certain bacteria; it cannot be isolated from living cultivations, but this can be effected after the micro-organisms have been poisoned with *alcohol*.

If we find a fairly loose connection with the body of the cell in the case of the ferments just mentioned, so that it is relatively easy to study them after separation from the vital activity of the parent cell, the *alcohol-producing* ferment of yeast, on the contrary, long resisted every attempt to isolate it. By the application of very violent measures, especially of great pressure, BUCHNER eventually succeeded in isolating from yeast the *enzyme* of *alcoholic fermentation*, *zymase*, which thus, to a certain degree, forms a connecting link between the yeast enzymes of the kind

¹ E. Fischer, *J. pr. Ch.*, xxvi., 77, 1898. For the remaining literature see under the different ferments in the special part.

mentioned above and the ferments which, as yet, have not been isolated at all. Its discovery gives us grounds for hoping that it may perhaps be possible by the application of similar vigorous means to also isolate the lactic acid and acetic ferments, which is a consummation to be desired as the crowning stone of our theoretical views.

In the case of all these ferments a direct observation of the process of secretion and of the changes of the parent cell which accompany it is naturally impossible, since a separation is only effected after so radical an injury to the protoplasm, that alterations in the histological sense can no longer be detected in the latter.

Hence the changes in the cells which excrete *readily soluble* ferments have been the more eagerly studied.

The enzymes of animals are, for the most part, produced and separated in special organs, which are termed *glands*. Thus there are the *salivary glands of the mouth*, which produce the saccharifying ferments, the glands of the *stomach* secreting *pepsin* and *rennet*, and the *pancreas glands*, which secrete the three groups of ferments, *trypsin*, *saccharifying* ferments, and *lipase*; besides these, though of less importance, we have the *intestinal glands* (whose most important product, in addition to *diastase*, is *invertase*), the *liver*, &c. In some of the lower animals the functions of several glands are united in the *gland* of the *intermediate intestine*, which, in addition to proteolytic, saccharifying, and fat-decomposing enzymes, sometimes also excretes enzymes which *dissolve cellulose*.

The histological alterations which these glands undergo during the secretion of the enzymes, as also the relations between the gland-cells and enzymes in general, have been most thoroughly studied and the most minute histological details settled, notably by the investigations of KÖLLIKER, ROLLET, HEIDENHAIN, V. WITTICH, GRÜTZNER, LANGLEY, BIEDERMANN,¹ and many others. It seems impossible to give a *résumé* of the result of these researches in a few words, and to follow them here into the more minute details would far exceed the limits of this work.

We will only notice here that the excreting cells during the *period of rest* are relatively large and contain little protoplasm, but are filled instead with substances partly more homogeneous and partly also of a granulated structure, which are regarded as ferments, or rather as the material for the formation of the ferments. If, now, the stimulus which induces secretion comes

¹ See the special part for references to the literature.

into play, these metaplasmatic substances are separated, the cell diminishes, and when a fresh period of rest ensues its protoplasm becomes capable of producing new supplies of ferments, which are first deposited in the cells in an inactive form as the so-called *zymogens*.

Whilst, in the case of animals, the histological problem was rendered far more definite by the existence of special glandular apparatus, it was otherwise in the case of the *plant-enzymes*. Long ago all kinds of *enzymes* were found to be widely distributed in plants, but as to their localisation in the special parts and tissues of the plants there was no agreement.

Only recently have the important conclusions of investigators begun to attract notice, in which they assume the presence of special organs of secretion or, at least, groups of secreting cells also in plants, and base these conclusions on histological grounds. These investigations will be described at length in the special part, and I will only refer here to those of L'ANGL, HABERLANDT, BROWN and MORRIS, and GRÜSS on diastase, of MARSHALL WARD on cytase, of GUIGNARD on emulsin and myrosin, and of GARDINER on the proteolytic ferments of *Dionæa*, all of whom attribute the production of enzymes to definite localised groups of cells, the secretory activity of which can be followed with the microscope.

Zymogens.—The more closely we study the subject of the secretion of soluble ferments the more probable does it appear that ferments in general are not present in the living cell immediately after the process of secretion in an effectively active condition, but in a transition stage, to which the name of *pro-ferment* or *zymogen* has been given. This has long been assumed in the case of *pepsin* and *trypsin*, and LANGLEY and HEIDENHAIN, in particular, have supported this view; the phenomenon, however, appears to be the rule in the case of a very large proportion of all enzymes, as has been pointed out, notably by GREEN.

These *zymogens* are in themselves inactive, but on contact with definite *zymoplastic* substances, notably dilute acids, they change into active ferments. As to the nature of this process of rendering the ferments active, we can only make suppositions; the fact that dilute acids are the best *zymoplastic* substances points to the conclusion that we may here be dealing with the decomposition of higher complex groupings, possibly consisting of compounds of the ferments with albuminous substances.

Zymogens, as a rule, offer more resistance than ferments to external influences.

It is frequently possible to render these zymogens accessible to a direct observation within the cell; they show *granules* of definite structure and colour, which are visible under the microscope. This is especially the case with the zymogens of *pepsin* and *trypsin* in their respective glandular cells; other observations, too, support this view, such as, for example, those of HABERLANDT on the diastase of the endosperm of seeds and of WARD on the cytase of the varieties of *Botrytis* examined by him.

CHAPTER VIII.

THE IMPORTANCE OF FERMENTS TO THE VITAL PROCESS.

ALTHOUGH we cannot go so far in our biological estimation of ferments as to venture to directly identify life with fermentative processes, yet the ferments play an extremely important part in the mighty circulation of solar energy, which underlies the entire metabolism of all living organisms. The substances of which the structure of plants and animals is built up are never offered to it in the form in which they are present in living protoplasm, but must before their absorption into the protoplasm invariably undergo a change which we may describe as *assimilation in the widest meaning of the term*.

This assimilation is, in the main, brought about by two processes—*synthesis* and *decomposition*. Both are essential functions of every living organism. The extent and physiological importance, however, of these different processes vary in the two great primary divisions of the organised world. Not that we are altogether justified in drawing a sharp line here, and definitely asserting that animals possess a pre-eminently destructive, and plants a pre-eminently constructive metabolism.

For the *biological importance* of a process does not depend upon its *course*. The *synthetic* construction of its protoplasm out of the plant-albumin conveyed to it in a suitable state of preparation or of its *glycogen* from foreign carbohydrates is just as indispensable to the animal, as are, on the other hand, *respiration* and the nourishment of the germ—processes accompanied by decomposition—to the plant. It is only when we compare the complete course of both processes, and measure it in arithmetical terms of the transferred energy, that we naturally come to the conclusion that a constructive endothermic metabolism, involving an expenditure of force, predominates in plants, whereas in the case of animals an exothermic metabolism predominates. And only in this sense can we retain the notion that the plant stores up the force brought to it in light and heat; whilst the

animal transforms this potential energy into kinetic energy—motion, heat, &c. It is true that the plant *alone* possesses the capacity inherent in its vital process and bound up with its chlorophyll system of evolving from simple substances completely devoid of energy, such as water, carbon dioxide, nitrogen, and inorganic salts, those complex substances which, in turn, afford a substratum for all further metabolic transformations. But apart from that, these substances show the same mode of transformation both in animals and plants. In the first place the *insoluble highly complex substances which are of no value for the cell*, such as proteins, starch (and cellulose) and fats are *broken up with the formation of simpler substances* (first phase), and these, according to their physiological destination, are transformed by a *fresh construction into the constituents of the protoplasm*,¹ or are further split up to give the kinetic energy required for heat, light, and electricity, &c.² (second phase). Whilst, now, this second class of construction appears in a specially pregnant form in the case of animals, but is of less importance in the case of plants, the *first phase*, on the other hand, is a process of decomposition into such products as can be formed into protoplasm by a new process of construction, and this new construction itself is a *biological process of equal importance* to plants and animals. In both we find the resolution of complex, non-utilisable substances into simpler compounds, and the assimilation of these simpler *soluble* compounds by the protoplasm.

Although we are now justified in assuming that the capacity for absorbing soluble substance, with a *fixation of energy*, is a prerogative of the *living cell*, and that *it alone* has the power of effecting such endothermic processes, yet *ferments* play a very important part in the *first phase*, the preparation *by means of decomposition* of the substances to be assimilated. It is primarily their work to induce exothermic decomposition

¹ From the protoplasm are then produced by *secondary* decompositions, which are in the main of a constructive character, all those chemical substances which have to fulfil some function or other of known or unknown character outside the protoplasm—bones, colouring matter of the blood, pigments, wood, ethereal oils, alkaloids, &c. These processes do not concern the problem before us.

² According to the view of Kassowitz (*Allg. Biol.*, Vienna, 1899) even *this* decomposition for the purpose of producing energy only takes place (*by metabolism*) *after* the absorption into the protoplasm. He thus assumes, speaking of it as a classification, that there is not a divergent subdivision of the *second* phase, but a simple absorption by the protoplasm, which is then followed by a *third* phase (the *decomposition* of the protoplasm).

processes which render complex nutritive substances soluble and capable of being absorbed by the protoplasm.

And so we need not be surprised at finding ferments wherever there is life. Passing upwards from the insignificant bacterium and the strange *Myxomycetes*, through the entire vegetable and animal kingdom, to the highly-organised flowering plants and the mammalia, we find everywhere their important activity. Whilst the various necessary ferments are mingled all together in the juices or sap of certain forms of life, we find in the higher organisms special organs to which they owe their production.

Thus unicellular and higher living organisms alike are capable, with the aid of their ferments, of so preparing the albuminous substances, carbohydrates, and fats which are offered to them as foods, that they become assimilable substances.

Let us consider, for example, the process in the case of a higher animal. The activity of the ferments of digestion begins even in the mouth; the non-utilisable starches are here partially converted into soluble sugar. This process stops immediately, or soon after, the food has been taken into the stomach. Here, on the other hand, commences the decomposition of the proteids by *pepsin*, which is assisted by the preparatory coagulation of milk by *rennet*. The most pronounced alterations of the foods, however, first occur in the *intestine*. Here the *fats* are decomposed, the decomposition of *albuminous substances* completed, and the *carbohydrates* which have been eaten, converted by various ferments into resorptionable mono-saccharides (glucose, galactose, fructose).

We must conceive a perfectly analogous process to occur in the case of plants. It is quite possible that plants build up synthetically a part of their assimilated substances from the simple food material only to the point at which it becomes directly resorptionable, producing, for example, *sugar* by direct synthesis; of this we are ignorant.¹

It is certain, however, that in periods of excessive production, when constructive processes develop in the sunlight, a large proportion of these processes continues to such an extent that higher products are formed, which are not assimilable without undergoing fresh decomposition, and these products the plant partially draws upon, with the aid of its ferments, even at periods of defective assimilation (*e.g.*, at night), for absorption into its cells or for the production of vital energy, or *stores them*

¹ The fact, however, that starch is only a reserve material—*i.e.*, that a large proportion of the sugar is used immediately after its synthetical formation, says much for this view—especially in the case of carbohydrates.

up in its reproductive organs. The embryo plant, which, when separated from the parent plant and thrown upon its own resources, must begin its new existence in a soil which affords it nothing except water and nutrient salts, is provided with a rich store of reserve food-materials, which are designed to support it until the formation of its own chlorophyll system insures its independent existence. These food-materials—albuminous substances, starch, cellulose, and fats—are, however, useless to the embryo in the form in which they are offered. In order to utilise them, the embryo plant makes use of ferments which convert them into serviceable substances.

A perfectly analogous course appears to be followed by embryo animals whose development commences in the egg, apart from the parent organism, as in the case of *birds*, &c.

It is a most interesting fact, and one which has been confirmed in the most divergent cases, that ferments are produced by the cell exclusively, or at any rate to a preponderating extent, when they are *required*—i.e., when no more food-material is available without further treatment.

Thus BROWN and MORRIS¹ explain the fact that the diastase-supply of leaves is greatest in the morning and decreases during the day, by the assumption that during the assimilation in sunlight, in which sugar is directly formed, the production of diastase *ceases* as superfluous; in fact, diastase is directly *destroyed* by light. (See *Diastase*.)

Moreover, the quiescent seeds of plants contain little or no ferment. The embryo has not yet come to life, and there is no need for the transformation of the stored-up reserve-material. But, as soon as germination commences, the necessary ferments are forthcoming. The embryo then forms for itself the saccharifying, proteolytic, fat-decomposing, and cellulose-decomposing ferments which it requires to render available the supplies with which it was endowed.² BROWN and MORRIS³ showed that the barley-embryo produced *no* diastase, when sugar, which it could absorb directly, was placed at its disposal. Speaking generally, diastase is only met with in plants in those parts in which starch “wanders;” in the case of potatoes, for instance, almost exclusively in the germinating tubercles and in the leaves (A. MAYER⁴).

In like manner the mould-fungi produce no ferments so long

¹ Brown and Morris, *Journ. Chem. Soc.*, lxiii., 604, 1893.

² *Vide, e.g.*, Hansen, *Arb. bot. Inst. Würzb.*, iii., 285.

³ Brown and Morris, *Journ. Chem. Soc.*, lvii., 395, 1890.

⁴ A. Mayer, *Chem. Centrbl.*, 1900, i., 824.

as they are grown on media from which they can directly supply their wants ; but they immediately develop *proteolytic* enzymes when they are cultivated on a culture-medium containing albumin, *diastase* when they are supplied with starch, and so on. *Moulds* grown upon culture-media containing tartaric acid and starch only develop diastase when the tartaric acid has been utilised. Naturally, *invertase* is also found in yeast which is grown in a solution of glucose.¹ According to AUERBACH,² bacteria grown on glucose do not form proteolytic ferments, and the same remark applies to *emulsin*, according to FERMI and MONTISANO.³

According to VAN TIEGHEM,⁴ the *Bacillus amylobacter* does not attack cellulose until no other source of carbon is placed at its disposal.

Very similar properties are possessed by *yeasts*, which can be accustomed to food substances for which they are not properly adapted ; they "learn," however, eventually to produce the suitable enzymes ; *Dienert*, in particular, has made interesting experiments of this kind. (See *Alcoholic Fermentation*.)

CL. BERNARD⁵ mentions an analogous instance in the animal world. The larvæ of *Musca lucilia*, a common fly, contain much glycogen, but no ferment that decomposes it (*diastase*). But, as soon as the larvæ pass into the stage of chrysalids, in which they consume the stored-up glycogen, a diastatic ferment is found in them.

And not only is the secretion of suitable enzymes developed by a physiological stimulus, but the *enzymes* themselves become adapted to the conditions of their environment. Thus it happens that enzymes of organisms which have specifically the same action, and which we group together under a common title, are yet found to vary with their different origin, especially when the organisms producing them exist under different conditions. Thus the various invertases, maltases, and emulsins show considerable differences in their behaviour towards acids, alkalies, salts, poisons, and changes of temperature. We will give the details when describing the individual ferments ; but may here just refer to two very characteristic cases.

The *pepsin* of the stomach of warm-blooded animals becomes

¹ Vide, e.g., Wortmann, *Z. ph. Ch.*, vi., 305.

² Auerbach, *Centralbl. f. Bact.*, 2nd Part, iv.

³ Fermi and Montisano, *Apoth. Ztg.*, 1894, 533.

⁴ Van Tieghem, quoted by De Bary, *Vorlesgn. üb. Bacterien*, Leipzig, 1885, 65.

⁵ Cl. Bernard, *Revue Scientif.*, 1873, 515, quoted by Schützenberger, *loc. cit.*, 254.

inactive as low as 10° C., whereas that of the *frog* is still active at 0° C.

In a perfectly analogous manner the *invertase* of *top-fermentation yeasts*, which are accustomed to relatively high temperatures, has a working *optimum*, which is about 25° C. higher than that of *bottom-fermentation yeasts*—a temperature at which the latter invertase is destroyed fairly rapidly.

Ferments, however, are formed not only when food-substances are actually *present*, which should satisfy the need of food, but also when there is the same physiological stimulus, but no means of satisfying it—i.e., in a *state of hunger*.

It has frequently been observed that mould-fungi are specially active in producing ferments when they have been deprived of food (FERNBACH¹).

They also use these, of course, to prepare the food-material stored up in their cells, but their development must without doubt, as a rule, be attributed, by parity of reasoning, to the want of directly assimilable food. They are thus produced to a certain extent in anticipation, so as to be able to immediately act upon any new supply of food.

The same explanation can also be offered for the fact that the digestive enzymes of higher animals, especially those of the stomach and pancreas, are found in the glands in the greatest abundance *at periods further removed from the last meal*, or in a fasting condition (GRÜTZNER, see *Pepsin*, &c).

We see then from all this what an important part is played by ferments in the vital economy of organisms, and we can only agree with NASSE² in regarding fermentative processes as an *essential part* of vital processes. Their main function is to render non-utilisable food-substances assimilable. Although it is also possible, as CHABRIÉ³ assumes, that the changes in osmotic pressure of the liquids, which must be formed during the breaking up and increasing of the molecules, play a part in the process, yet it cannot be a very considerable one.

I must, however, here again lay stress upon the point that important as are the processes for the vital process, yet they have only a *subordinate* significance; and although the organism cannot exist without them they must nevertheless in theory be rigorously separated from *specific vital* processes, inasmuch as the same phenomena can be produced apart from life in a test-tube. In spite of the biological importance of the enzymic actions, they must certainly not be regarded as biological any

¹ Fernbach, *Ann. Inst. Pasteur*, 1890, 1.

² Nasse, *Pflüg. Arch.*, xi., 163. ³ Chabrié, *C. R. Soc. Biol.*, 1898, 105.

more than the circulation of water, which is equally a fundamental biological condition for all organisms, can be spoken of as a phenomenon of the vital process.

Distribution of Ferments in Nature.—We have already briefly referred to the universal distribution of ferments. In fact, they can be detected everywhere in the organised world, so that we can regard them as a constant concomitant phenomenon of life.

In the lowest organisms, *the bacteria*, ferments of the most diverse kind are found, particularly proteolytic, diastatic, and (more rarely) inverting ferments (FERMI¹). KRUKENBERG discovered a proteolytic ferment in *Fuligo septica*; in yeasts there are undoubtedly present, in addition to *zymase*, all the saccharifying ferments, as well as proteolytic ferments. In the *mould-fungi* and also in higher fungi numerous ferments have been discovered, notably through the researches of BOURQUELOT and his pupils; in the sap and organs of higher plants, in their leaves, flowers, &c., they are found not less widely distributed.

They have been systematically sought for in the organs of lower animals, notably by KRUKENBERG, and in insects they have been found by BASCH, ERLÉNMEYER, BIEDERMANN, and others.

Fishes, again, have been examined for ferments by KRUKENBERG, who has been able to detect them both in the mouth and intestines.

In amphibia ferments were found by FICK and others.

The ferments of *mammalia* have naturally been the most fully investigated. Ferments have been found in them, not only in the organs specially adapted for their production, but distributed throughout the body in all the tissues and juices (of tissues). The saccharifying ferments, in particular *diastase*, &c., are almost ubiquitous in their occurrence in the tissues, since they have been found not only in the spleen, kidneys, liver, &c., but also in the blood, lymph, and secretions, *urine*, *sweat*,² &c. They could also be detected in pathological growths, in the sputum of those suffering from lung diseases (FILEHNE), in exudations (EICHHORST), and in the fluid from cysts (v. JAKSCH).

They are also found widely diffused in the *growing* organism. The biological significance of the ferments of germinating seeds has already been mentioned. The *diastase* of germinating barley was the first generally recognised diastatic ferment (PAYEN and PERSOZ). Subsequently *proteolytic* (by GORUP-BESANEZ), fat-decomposing, glucoside-decomposing, and other ferments were

¹ The bibliography is given in the special part.

² * Gaube, *C.R. Soc. Biol.*, 1891, 115.

also found in seeds. The existence of proteolytic and diastatic enzymes in the *hen's egg* is very probable.

Moreover, ferments have also been discovered in the embryos of animals and of man. LANGENDORFF¹ has closely investigated this question. He found them in all the embryonic mammalia and birds which he examined, although characteristic differences appeared. Whilst *trypsin* could be detected in all at a very early stage, *pepsin* was altogether absent before birth in carnivora, though it was formed at a relatively early period in herbivorous animals. *Diastase* was found at an early stage in the pig, rat, and ox, but did not occur before birth in man or in rabbits. The first occurrence of ferments in general in the embryo of the pig was observed when the animal had attained a length of 120 mm. As regards the development of the ferments of the pancreas, DAHL² has established the fact that *trypsin* is formed first, then *lipase*, and finally *diastase*.

The Fate of Ferments in the Organism.—The normal ferments of the organism are partially resorbed in the course of their activity into the intestines, and pass in small quantities into the *urine*. Another part is excreted with the *fæces*. GEHRIG³ and SCHNAPPAUF,⁴ however, support the view that complete active *pepsin* does not pass into the circulation, or if so only to a small extent, but is rather resorbed directly from the *gland* as *zymogen*. Moreover, a large proportion is *not* separated from the body; it is thus either destroyed somewhere or other in the organism or withdrawn again into the secreting glands.

In the *urine* *pepsin*, *diastase*, and *rennet* have been identified with certainty; the occurrence of *trypsin* under normal conditions is doubtful.

A large part of the ferments, however, is rendered *at least* inactive in the digestive canal; but whether or not they are *destroyed* cannot be definitely stated.

Through the researches of LANGLEY⁵ it has been proved that although saliva-diastase remains active in the stomach for a considerable time, yet it finally becomes completely inactive. The pepsin and rennet of the gastric juice are rendered inactive or destroyed by the alkaline intestinal secretions; this is a physiological necessity, since otherwise the pepsin would interfere to

¹ Langendorff, *Du Bois A. f. Phys.*, 1879, 95. (Contains a complete bibliography of the older literature.) See also in the special part.

² Dahl, *Diss. Dorpat*, 1890, quoted in *Centralb. f. Physiol.*, 1891, 309.

³ Gehrig, *Pflüg. Arch.*, xxxviii., p. 35, 1886.

⁴ Schnappauf, *Beitr. Physiol. des Pepsins*, Diss. Rostock, 1888.

⁵ Langley, *Journ. of Physiol.*, iii., 246.

a great extent with the action of the trypsin. KÜHNE also assigns an important function in rendering the pepsin inactive to the acids of the gall, and explains the digestive disturbances which are produced by a stoppage of the gall-ducts leading to the intestine (in jaundice, &c., or by fistulas) as partly due to the cessation of this function.

Trypsin and the other intestinal ferments are asserted by Langley to be destroyed by the acids formed in the putrefactive processes in the intestine.

The organism, however, must have the means of disposing of ferments which are introduced into it in an unusual way. To what extent they are destroyed, and to what extent, on the other hand, they are only rendered inactive or, perhaps, converted to their physiological function again, we are unable to form a conclusion.

Thus BÉCHAMP and BALTUS¹ observed that when diastase was injected into the veins it only passed partially into the urine.

SCHNAPPAUF² could not detect any increase in the normal amount of pepsin in the urine, after the subcutaneous injection of *pepsin*.

HILDEBRANDT³ was able to prove that emulsin subcutaneously injected was *not, as a rule*, excreted with the urine. The organism thus completely destroys a foreign ferment. By the fact that the introduction of *amygdalin* caused prussic acid poisoning so long as emulsin was present in the body, HILDEBRANDT was able to show that six hours after the injection a sufficient quantity of the ferment was still present to cause the poisoning of the rabbit used in the experiment. He was also able to prove that the ferment passed into the blood, but was destroyed more rapidly there. Active ferment was also present in the spleen, pancreas, and specially the *liver*, as also notably in the connective tissue and the lymph glands near the point of injection. The ferment of the parenchymatous organs had no action upon the amygdalin circulating in the blood, and thus appeared to be attached to the *cells*, a fact of great interest for the explanation of the *poisonous action* of ferments mentioned above. The observed injurious action of the blood-serum on ferments *in vitro* was also found by Hildebrandt to hold good in the case of rennet within the living animal. From all these results it follows that ferments under all conditions are to a great extent destroyed in the organism.

¹ Béchamp and Baltus, *C.R.*, xc., 373, 539.

² Schnappauf, *Beitr. z. Physiol. und Pepsins*, Diss. Rostock, 1888.

³ Hildebrandt, *Virch. A.*, cxxxi., 12, 1893.

SPECIAL PART.

A. THE HYDROLYTIC FERMENTS.

CHAPTER IX.

THE PROTEOLYTIC FERMENTS.

THE proteolytic ferments possess the power of decomposing albuminous substances in a definite manner, and converting them into simpler substances. As to the nature and method of these processes nothing definite can be asserted, since the constitution not only of the genuine albuminous substances, but also of some of the decomposition products is very obscure. We have therefore to be content with isolating the final products of these fermentative processes, and determining as far as is possible their chemical nature.

The proteolytic enzymes are divided into *three main groups*. The enzymes in the first group, of which the most important representatives are the *pepsin* peculiar to the stomach of vertebrate animals, and the ferments analogous to it, act very energetically upon the albumin molecule; they produce substances of as yet unknown nature—*albumoses* and *peptones*. As a rule, they are only active in dilute acid solutions. The enzymes of the second main group, represented by *trypsin*, which are, moreover, active in neutral or dilute alkaline solutions, decompose the albumin molecule in a very energetic manner, almost corresponding to the decomposition effected by strong chemical agents; the products which result are relatively simple, are for the most part of known composition, and have been synthetically prepared. They consist, in the main, of nitrogenous substances, such as *amido acids* and the so-called *hexone bases*. A sub-group of these proteolytic ferments stands

midway between the two preceding groups. Its most important representative is *papain*. The *third* group of the albumin-changing ferments contains the *coagulating* enzymes, which probably also act hydrolytically, and to which belong *rennet* and the hypothetical *fibrin ferment*. The coagulating ferments also include the but little investigated *pectase*. Naturally the proteolytic ferments play an important part in the metabolism of animals and also of plants. In animals they are a secretion of the digestive glands; they are accordingly found in the liquid of the digestive tract; the ferments, of which pepsin is the type, having their seat in the *stomach* and performing there to a certain extent the preliminary work, whilst subsequently the *cryptic* ferments of the *pancreas* continues the further decomposition in the intestine. Speaking generally, later investigations show that the proteolytic enzymes are extremely widely distributed not only in the animal kingdom, but also throughout the vegetable kingdom.

According to FERMI¹ *pepsin* is to be regarded as only a modification of *trypsin*, caused by the acidity of the medium, and subsequently appearing phylogenetically as *trypsin*. As against this view, however, we have not only the totally different activity of the two ferments, but also, as far as our scanty results go, their material difference.

In general, they are stated to only act upon *dead protoplasm*, whilst they leave living cells intact. FERMI¹ states that living *Amœbæ*, *Schizomycetes*, and moulds are not attacked by pepsin. In his opinion this resistance of the living protoplasm to the ferments of digestion affords the explanation why the living mucous membrane of the stomach does not digest itself, whereas immediately after death the auto-digestion commences. From this point of view he vigorously attacks the statements of HILDEBRANDT and KIONKA as to the deleterious action of ferments on living tissue (see *General Part*), yet the latter appear to be right in regarding ferments as actually violent poisons to the protoplasm, and their conclusions apparently deal a heavy blow against Fermi's theory of the resistance of the living mucous membrane to pepsin.

Pepsin.—The fact that an important part in digestion is assigned to the stomach was known to the physicians of old; at a very early period it was perceived that the acid of the stomach alone was not sufficient to digest food-substances, but that, in addition to this, a *ferment*, a substance similar to the active

¹Claudio Fermi, *Arch. ital. d. Biolog.*, 1895, 433. Cf. *Centralb. f. Physiol.*, viii., 657; ix., 57, 1895.

principle of alcoholic fermentation, co-operated (VAN HELMONT¹). The importance of the gastric juice of the glands of the stomach was first recognised by BORELLI. RÉAUMUR² then proved that gastric digestion was independent of the mechanical power of the stomach, but that the food-substances underwent rather a chemical change through the influence of the *gastric juice*, and established the solvent action of the gastric juice on vegetable foods. Next came the classical investigations of SPALLANZANI,³ who clearly demonstrated the difference between the phenomena of alcoholic fermentation, putrefaction, and gastric digestion, and was the first who was able to show digestive processes *outside* the body. At this point, then, begins the history, properly so-called, of the *peptic ferment*, which now, for the most part, proceeds in close connection with the history of gastric digestion. BEAUMONT investigated the processes in the stomach by direct observation in a case of traumatic fistula of the stomach; PROUT and TIEDEMANN and GMELIN discovered independently the *hydrochloric acid* in the stomach.

An important step towards the discovery of the ferment was made by EBERLE,⁴ who was the first to prepare artificial gastric juice, and thus gave rise to the classical work of SCHWANN,⁵ who attributed gastric digestion to a *ferment*, to which he gave the name of *pepsin*. He showed that this ferment did not belong, as Eberle had assumed, to the mucus, therefore being present in all mucous membranes, but that it was exclusively a product of the mucous membrane of the stomach. Schwann did not succeed in isolating the ferment itself, nor, up to the present time, has anyone been successful in separating pepsin as a chemically pure substance, so that its properties can only be studied in approximately pure preparations. Schwann himself had obtained a preparation containing pepsin by precipitation with mercuric chloride and decomposition of the precipitate; WASMANN⁶

¹ For the older literature on our knowledge of gastric digestion, see, *inter alios*, Gamgee, *Physiologische Chemie der Verdauung*, translated into German by Asher and Beyer. Leipzig and Vienna, 1897, 61, &c.

² Réaumur, *Mém. de l'Acad. des Sciences*, 1752, 461.

³ Spallanzani, *Versuche üb d. Verdauungsgeschäft*. German translation by Michaelis. Leipzig, 1785.

⁴ Eberle, *Physiol. d. Verdauung auf natürlichem und künstlichem Wege.*, Würzburg, 1834.

⁵ Schwann, *Müller's Archiv.*, 1836, 90. Cf. Joh. Müller and Schwann, *ibid.*, 66, and the dissertation by Gerson, Berlin, 1835, *De Chymificatione artificiosa*, quoted there.

⁶ Wasmann, *De digestionem animalis*, Diss., Berlin, 1839. Quoted *verbatim* in Hermann's *Handbuch d. Physiol.*, v., Part ii., 44.

obtained a solid active preparation by a similar proceeding, and precipitation of the filtrate with alcohol. By mechanical precipitation with freshly precipitated calcium phosphate, dissolving the precipitate, re-precipitating with *cholesterin* and extracting the *cholesterin* with ether, BRÜCKE¹ obtained a solution of pepsin, which, like the others, was not pure. Subsequently, v. WITTICH² made use of the property possessed by most enzymes of dissolving in glycerin for the preparation of pepsin solutions, from which he could precipitate the pepsin with alcohol. To purify it he also employed *dialysis*, following the example of KRASSILNIKOFF,³ since pepsin does not diffuse through animal membranes and parchment paper. To prevent putrefaction, it is necessary to add thymol or to keep the dialysed liquid acid. In like manner MALY,⁴ by precipitation with calcium phosphate and dialysis, prepared a solution of pepsin. Other methods have been employed by LEHMANN,⁵ C. SCHMIDT,⁶ FRERICHS,⁷ and others. By simply cooling gastric juice to 0° C., Frau SCHOUMOW-SIMANOWSKI⁸ obtained a solid product, which represented pepsin similarly contaminated to a very great extent with albuminous substances. In a similar manner, by dialysing fresh gastric juice, PEKELHARING⁹ obtained a body of a nucleo-proteid character, which was stated to be an exceedingly active *pepsin*. It split up into a substance containing phosphorus and an albuminous body. In Pikelharing's opinion, pure *pepsin* must be regarded as possessing a nature resembling that of a nucleo-proteid.

The Secretion of Pepsin.—There has been much controversy on the question of the source from which pepsin is produced. The main points discussed are whether only the *fundus glands* of the stomach produce pepsin, or also the so-called *mucous glands* of the *pylorus*; further, which cells of the fundus glands are the place where the pepsin is formed. One group of investigators supports the view that the *chief cells* of the fundus glands are physiologically homologous with the mucous glands, and that both effect a secretion of pepsin, whilst only the production of

¹ Brücke, *Vorlesg. üb. Physiol.*, 1874, i., 294.

² v. Wittich, *Pflüg. A.*, ii., 193; iii., 339.

³ v. Wittich, *ibid.*, v., 435.

⁴ Maly, *Pflüg. A.*, ix., 592.

⁵ Lehmann, *Ber. d. sächs. Ges. d. Wiss.*, 1849, 10.

⁶ Bidder and Schmidt, *Verdauungssäfte*, 45.

⁷ Frerichs, *Verdauung*, iii., 782; quoted by Brücke, *loc. cit.*

⁸ Schoumow-Simanowski, *A. exp. Path.*, xxxiii., 336.

⁹ Pikelharing, *Z. f. physiol. Ch.*, xxii., 1896-97.

acid is assigned to the parietal cells (HEIDENHAIN,¹ FICK,² and specially EBSTEIN³ and his pupils).

The others, on the contrary, regarded the parietal cells as the seat of the formation of pepsin (EDINGER⁴) in fish (NUSSBAUM⁵), or at least denied any part in the process to the pylorus glands (KÖLLIKER,⁶ DONDERS,⁷ FRIEDINGER,⁸ WOLFFHÜGEL,⁹ and, above all, v. WITTICH¹⁰). Nussbaum (*loc. cit.*), it is true, admitted that the region of the pylorus might generate pepsin, but contended that even there the parietal cells were the pepsin-producers. He further pointed to the fact that in hibernating animals the parietal cells partially disappeared when digestion ceased. v. WITTICH had at first obtained an inactive glycerin extract from the region of the pylorus, and when EBSTEIN showed that the pylorus region by itself produced a secretion containing pepsin, Wittich and his co-workers replied that the pylorus part did not in itself contain pepsin, but absorbed it from the fundus glands; and that thus the pepsin appeared in the extracts from the pylorus. Even now this point has not been settled beyond doubt, although there is much to be said in favour of Ebstein's view. Important results were obtained from the examination of the stomach of *frogs*. v. SWIECICKI¹¹ found that there the pepsin was mainly formed in the *alimentary canal* by glands which were analogous to the chief glands of the stomach, whilst in the stomach itself an acid secretion was formed by glands similar to the parietal cells—which was altogether denied by FRÄNKEL.¹² SEWALL¹³ found only *parietal cells* in the fundus glands, and *no* pepsin in the case of *young* embryos of the sheep; not till a later period did the *chief cells* also appear, and then pepsin was found, but otherwise he assumed that the pylorus produced no pepsin. KLEMENSIEWICZ¹⁴ succeeded by means of an operation in obtaining from the living

¹ Heidenhain, *A. f. mikr. Anatomie*, vi., 368.

² Fick, *Verh. d. Würzb. phys. med. Ges.*, 1872, 121.

³ Ebstein, *A. f. mikr. Anat.*, vi., 515; Brunn and Ebstein, *Pflüg. A.*, iii., 565; Ebstein and Grützner, *Pflüg. A.*, vi., 1; *ibid.*, viii., 122, 617; xvi., 105.

⁴ Edinger, *A. f. mikr. Anat.*, xiii., 651.

⁵ Nussbaum, *A. f. mikr. Anat.*, xiii., 721; xv., 119; xvi., 532.

⁶ * Kölliker, quoted by Ebstein, *loc. cit.*

⁷ Donders, *Physiol.*, 1856, 208.

⁸ Friedinger, *Sitzb. d. Acad. d. Wiss. Wien*, 1871, 325.

⁹ Wolffhügel, *Pflüg. A.*, vii., 188.

¹⁰ v. Wittich, *Pflüg. A.*, v., 434. vii., 18; viii., 444.

¹¹ v. Swiecicki, *Pflüg. A.*, xiii., 444, 1876.

¹² Fränkel, *Pflüg. A.*, xlviii., 63. ¹³ Sewall, *Journ. of Physiol.*, i., 321.

¹⁴ Klemensiewicz, *Sitzb. d. Acad. d. Wiss. Wien*, lxxi., Part iii., 24.

animal pure pylorus juice, which had an *alkaline* reaction, and after acidification with hydrochloric acid showed *peptic activity*.

HEIDENHAIN¹ has confirmed these results, as has also ÅKER-MANN,² whilst CONTEJEAN³ contests them.

Finally, mention should also be made that according to FICK⁴ and NUSSBAUM⁵ the pepsin of the pylorus tract behaves very differently to that of the fundus glands, producing much more *parapeptone* (*vide infra*) in an analogous manner to the *isopepsin* of Finkler (*vide infra*).

The secretion of pepsin by the stomach is not perfectly constant, but varies considerably.

Normally it depends upon the introduction of food, but the rate of the conversion of pepsinogen into the active ferment is influenced by the acidity of the gastric juice. ROTH⁶ draws a distinction between *hyperpepsia*, in which abnormal amounts of ferment are present, and *hypopepsia*, in which there is a very sparse occurrence of ferment. He found the former specially in cases of *ulcus ventriculi* and the latter in *carcinoma* and *chronic gastritis*.

Pepsinogen.—EBSTEIN and GRÜTZNER⁷ had already shown that it was probable that the actual secretion of the glands of the stomach was not pepsin itself, but an unstable intermediate compound. They found that this intermediate substance was not extracted by glycerin, so that glycerin extracts of the mucous membrane of the stomach were less active than those made with hydrochloric acid, which converts *pepsinogen* into pepsin. Somewhat similar results were obtained by CHAPOTEAUT⁸ and PODWISSOTSKI.⁹

LANGLEY¹⁰ then gave a convincing proof that such a zymogen actually existed and that it was directly visible in the granules of the *chief cells*, and also that the glands of the stomach contained no ferment during life, but *only* the zymogen. They are mainly distinguished from one another in their behaviour towards dilute alkalis and carbonic acid (*vide infra*). Dilute acids and the introduction of carbonic acid transform pepsinogen very rapidly into pepsin.

¹ Heidenhain, *Pflüg. A.*, xviii., 169.

² Åkermann, *Skand Arch. f. Physiol.*, v., 134, 1895.

³ Contejean, *A. d. Physiol.* [5], ix., 554.

⁴ Fick, *Verh. Würzburg phys. med. Ges.* new series, ii., 122, 1872.

⁵ Nussbaum, *Arch. f. mikr. Anat.*, xiii., 721, &c.

⁶ Roth, *Z. f. klin. Medic.*, xxxix., 1, 1900.

⁷ Ebstein and Grützner, *Pflüg. A.*, viii., 127.

⁸ Chapoteaut, *C.R.*, 1882, xcix., 1722.

⁹ Pódwissotski, *Pflüg. A.*, xxxix., 62.

¹⁰ Langley, *Journ. of Physiology*, iii., 269.

BÉCHAMP¹ sees in these granules his remarkable *microzymata*, which he regards as organised forms. For further particulars of these reference must be made to his original work.

LANGLEY and EDKINS² have subsequently given a method for the separation of pepsin and pepsinogen.

Pepsin is found in the stomach of almost all vertebrate animals; only in the case of certain fishes is it wanting. With reference to the digestion of fishes, investigations have been published by KRUKENBERG³ and by RICHET.⁴ KRUKENBERG found more pepsin in the upper segment of the intestine, and more of a secretion of a trypsin character in the lower intestine.

In certain herbivora—*e.g.*, *rabbits*—it occurs even in the fœtus, whilst in carnivora it is absent at birth.

In *invertebrate animals* enzymes of the nature of pepsin are also found. BASCH⁵ has discovered a product acting in a perfectly analogous manner in the salivary glands of the common kitchen cockroach (*Blatta orientalis*).

KRUKENBERG⁶ has examined numerous invertebrates of the most different classes with reference to their digestive enzymes. He only failed to discover them completely in the *Cœlenterata*; in other cases he generally found them. (Summary *loc. cit.*, p. 363). He also found pepsin in the yolk of egg⁷ in which peptones had also been found previously.

As regards digestion in insects, researches have been made notably by PLATEAU, FRENZEL, JOUSSET DE BELLESME, BOUCHARDAT,⁸ and HAYER.⁹ A thorough investigation of the digestive enzymes of *Tenebrio molitor* (meal-worm) has been made by BIEDERMANN.⁸

Outside the stomach *pepsin* has been detected in Brunner's glands (GRÜTZNER),¹⁰ whereas the proteolytic ferments of the *intestinal mucous membrane* were entirely absent.¹¹ It has, more-

¹ Béchamp, *Comptes Rendus*, xciv., 1882, 582, 879, 970. Cf. Gautier, *ibid.*, 9, 652.

² Langley and Edkins, *Journ. of Physiol.*, vii., 371.

³ Krukenberg, *Unters. a. d. physiol. Inst. Heidelberg*, ii., 385.

⁴ Richet, *Archives de Physiol.* x., 1882, 536.

⁵ Basch, *Sitzb. Wiener, Acad.*, xxxiii., 1858, *Math. Nat. Cl.*, 255.

⁶ Krukenberg, *Unters. a. d. physiol. Inst. Heidelberg*, ii., 1, 37, 261, 338, 366, 402.

⁷ Krukenberg, *ibid.*, 273.

⁸ Biedermann, *Pflüg. Arch.*, lxxii., 160; references.

⁹ Hayer, *Note additionelle sur la digestion chez les ins.*, Brussels, 1877; *Z. physiol. Ch.*, ii., 208.

¹⁰ Grützner, *Pflüg. Arch.*, xii., 288.

¹¹ Of recent work see Wenz, *Z. f. Biol.*, xxii., 1; Pregl, *Pflüg. A.*, lxi., 359; cf. Gamgee, *Phys. Chem. d. Verdauung*, 422.

over, been found in the *urine* (by BRÜCKE,¹ POEHL,² who only found it in mucilaginous urine, TASULLI,³ GRÜTZNER,⁴ SAHLI,⁵ GEHRIG,⁶ STADELMANN,⁷ HOFFMANN,⁸ and BENDERSKY;⁹ and in pathological urines by MYA and BELFANTI¹⁰; also in the muscles (BRÜCKE), in the saliva (MUNK¹¹), and so on.

In diseases of the digestive tract it is said to be absent from the urine (LEO¹²). It has been found by BENDERSKY¹³ in sweat, together with diastase, whilst trypsin is said to be absent. Peptonising agents, possibly to be attributed to a ferment resembling pepsin, were found by POEHL¹⁴ in the *kidneys, lungs*, and, to a less extent, in the *intestinal tissue*. HAHN and GERET¹⁵ found a proteolytic ferment in the juice obtained from the tissue of the liver by Buchner's method, but it is questionable whether this ought not rather to be assigned to the ferments of the nature of trypsin.

Properties of Pepsin.—The different preparations which are found in commerce under the name of *pepsin* differ according to their origin and preparation, both in properties and activity.

KONOWALOFF¹⁶ has tested the activity of various commercial peptones as compared with that of the pure gastric juice of a dog. A further examination of commercial pepsins (28) has been made by VENTURINI and COTTA.¹⁷

It appears, however, that, apart from their degree of purity, the ferments behave differently, not only in the case of different species of animals, but also in the case of the same animal—i.e., as regards their activity in different acids. The pepsin of the *dog* is the most active (KLUG,¹⁸ WRÓBLEWSKI¹⁹). Moreover, FICK²⁰

¹ Brücke, *Vorlesg. üb. Physiol.*, 1874, i., 295.

² Poehl, *Ueb. das Vork. u. d. Bildg. des Peptons ausserhalb des Verdauungs-apparats*, &c., Diss. Dorpat., 1882; *Biol. Centralbl.*, iii., 252. See also *B. d. d. chem. Ges.*, xiv., 1355.

³ Tasulli, *Maly's Jb.*, 1894, 289.

⁴ Grützner, *Breslauer ärztl. Ztschr.*, 1882, 17.

⁵ Sahli, *Pflüg. Arch.*, xxxvi., 209.

⁶ Gehrig, *ibid.*, xxxviii., 35, 1886.

⁷ Stadelmann, *Z. f. Biol.*, xxiv., 266.

⁸ Hoffmann, *Pflüg. Arch.*, xli., 148.

⁹ Bendersky, *Virch. A.*, cxxi., 554.

¹⁰ Mya and Belfanti, *Gazzetta degli Ospitali*, 1886, 3, quoted in *Centralbl. f. klin. Med.*, 1886, 449, 729.

¹¹ * Munk, *Verh. d. physiol. Ges. Berlin*, 24 Nov., 1876.

¹² Leo, *Pflüg. Arch.*, xxxvii.

¹³ Bendersky, *Virch. A.*, cxxi., 554.

¹⁴ Poehl, *Ueb. das Vork. u. d. Bildg. des Peptons*, &c., as above.

¹⁵ Hahn and Geret, *Ber. d. d. chem. Ges.*, xxxi., 2335, 1898.

¹⁶ Konowaloff, *Maly's Jb.*, 1894, 289; from a diss., Petersburg, 1893.

¹⁷ Venturini and Cotta, *Bollet. Chim. Farm.*, xxxix.; *Chem. Centralbl.*, 1900, i., 619.

¹⁸ Klug, *Pflüg. A.*, lx., 43, 1895.

¹⁹ Wróblewski, *Z. f. physiol. Ch.*, xxi., 1895.

²⁰ Fick, *Verh. d. Würzb. phys. med. Ges.*, 1873, 121.

states, on the authority of experiments by MURISIER, that the pepsin of the frog is active even at 0° C., whilst that of warm-blooded animals shows no activity below 10° C. According to later researches by KLUG,¹ however, the pepsin of warm-blooded animals is also still active at 0° C., although but slightly so.

Properties of Pepsin and Pepsinogen.—*Pepsin*, which, as was mentioned above, is not yet known in the pure condition, is a substance of high molecular weight related to the albuminous bodies, though of completely unknown chemical constitution.

The following analysis of pepsin is by SCHMIDT² :—

C = 53.0 per cent.	N = 17.8 per cent.
H = 6.7 „	O = 22.5 „

It shows, in general, the usual properties of an enzyme. In its purest known condition it appears as a white or light yellow amorphous mass. It is soluble in water, dilute solutions of salt, dilute acids, and glycerin; it is precipitated from its solution by an excess of alcohol.

A solution of Brücke's pepsin (*vide supra*) gives no precipitate with the ordinary reagents for proteids, such as platinum chloride, mercuric chloride, tannin, and nitric acid; but, on the other hand, is precipitated by normal and basic *lead acetate*. It gives a faint xanthoproteic reaction. SUNDBERG,³ too, could not observe any albumin reactions in his pepsin preparations (*cf.*, however, PEKELHARING, *supra*).

Heating its solution to 55° to 57° C. destroys the activity, as in the case of all enzymes (see, *inter alios*, MAYER⁴); hydrochloric acid, certain salts, and also peptones raise the temperature of decomposition.⁵ In the dry state it resists heating up to 100° C.

TINKLER⁶ also asserts that, by gently warming at 60° C., he has effected a conversion of pepsin into *iso-pepsin*, which, in the digestion of albumin, yields greater quantities of *parapeptone* (anti-albuminate, *vide infra*) than does pepsin. We have here, doubtless, an incomplete decomposition of the pepsin.

It is absolutely non-diffusible through parchment paper (HAMMARSTÉN,⁷ WOLFFHÜGEL⁸), but diffuses through De la Rue's paper (FERMI and PERNOSI⁹).

As is the case with most enzymes, it is mechanically carried

¹ Klug, *Pflüg. A.*, lx., 65.

² Bidder and Schmidt, *Verdaunungssäfte*, Milan and Leipzig, 1852, 46.

³ Sundberg, *Z. physiol. Ch.*, ix., 319.

⁴ Mayer, *Z. f. Biol.*, xvii., 351, 1881.

⁵ Biernacki, *Z. f. Biol.*, xxviii., 62. ⁶ Tinkler, *Pflüg. A.*, xiv., 128.

⁷ Hammarstén, *Maly's Jb.*, iii., 160. ⁸ Wolffhügel, *Pflüg. A.*, vii., 188.

⁹ Fermi and Pernossi, *Z. f. Hyg.*, xviii., 105, 111.

down by falling precipitates, especially by precipitates of albumin, but also, *e.g.*, by calcium phosphate.¹

It combines with hydrochloric acid to form a loose compound (v. WITTICH²).

In like manner it enters into combination with fibrin, so that it cannot be separated again by extraction with glycerin (v. WITTICH, EBSTEIN, and GRÜTZNER³). This ready combination is used for the detection of traces of pepsin (*e.g.*, in urine).

Pepsin is very sensitive to the action of alkalies. HERZEN⁴ states that alkalies render it inactive, but that carbonic acid restores its activity, which, however, is denied by FERMI and PERNOSI.⁵ According to KÜHNE⁶ and LANGLEY,⁷ pepsin is very rapidly destroyed by a 0.5 to 1 per cent. solution of soda, whilst, on the other hand, *pepsinogen* is fairly stable, or, rather, is destroyed much more slowly. Trypsin, according to KÜHNE,⁸ does not attack pepsin in a neutral solution. LANGLEY⁹ finds that trypsin accelerates the decomposition of pepsin by alkalies; it also acts upon the zymogen. A similar statement is made by MEES.¹⁰

The pepsin of the *frog* is more stable. In the presence of albuminous bodies the destruction of pepsin is retarded. It is gradually decomposed, even by carbonic acid. Pepsinogen is equally soluble in water and hydrochloric acid, but less soluble in glycerin when the latter does not contain much water. At about 55° C. it loses its activity. Dilute acids and carbonic acid rapidly convert it into pepsin. Certain salts accelerate this transformation, whilst proteids, and specially peptones, retard it.

THE ACTION OF PEPSIN.

I. Methods of Identifying and Determining the Activity.

We have no other means of identifying pepsin than the determination of its activity on albuminous bodies. A dilute solution of pepsin containing 0.1 to 0.2 per cent. of hydrochloric acid is allowed to act upon fibrin or egg-albumin, and the

¹ Brücke, *Vorlesg. üb. Physiol.*, 1874, i., 294.

² v. Wittich, *Pflüg. A.*, 203. ³ *Loc. cit.*

⁴ Herzen, *Annali di Chim. e. Farm.*, viii., 304, 1888.

⁵ Fermi and Pernossi, *Z. f. Hyg.*, xviii., 105, 111.

⁶ Kühne, *Verh. naturh. med. Ver., Heidelberg*, 1877, 193.

⁷ Langley, *Journ. of Phys.*, iii., 246. ⁸ Kühne, *loc. cit.*

⁹ Langley, *loc. cit.* ¹⁰ Mees, *Maly's Jb.*, 1885, 269.

arrosion (digestion) of the albumin—*i.e.*, the amount dissolved—subsequently determined, and the decomposition products afterwards detected in the filtrate.

In order to measure quantitatively the activity of pepsin the amount of undigested albumin is either weighed after a definite time of action (BIDDER and SCHMIDT¹ or the method of GRÜNHAGEN² is employed. In this, fibrin is saturated with pepsin in hydrochloric acid, and the mass transferred to a filter. In proportion as the fibrin becomes liquefied the drops fall from the funnel, and the number of drops falling in a definite time affords a measure of the activity of the pepsin.

An ingenious modification of this method, which makes it suitable, for instance, for a lecture demonstration has been proposed by GRÜTZNER.³ He colours the fibrin to be placed on the filter with *carmine*, so that the liquid then running through has a red colour, owing to the pepsin causing the red colouring matter to pass into solution with the fibrin. The reliability of this method has been called in question by KLUG,⁴ who proposes instead a spectro-photometric estimation of the intensity of the biuret reaction. This method has since been modified by METTE.⁵ SCHÜTZ⁶ estimates the peptones polarimetrically; SCHIFF⁷ by determining the specific gravity of their solutions.

METT⁸ places small glass tubes, 1 to 2 mm. in diameter, filled with coagulated albumin, in the liquid under examination, leaves the whole for ten hours in the incubating oven, and measures the length of the column of albumin dissolved.

II. Influence of External Factors.

The results of the investigations, so far as they concern the activity of pepsin are as follows:—

Pepsin is not used up in digestion, nor does it increase in quantity (BRÜCKE).⁹

¹ See Ebstein and Grützner, *Pflüg. Arch.*, vi., 1.

² Grünhagen, *Pflüg. Arch.*, v., 203.

³ Grützner, *Pflüg. Arch.*, viii., 452.

⁴ Klug, *Pflüg. Arch.*, ix., p. 43.

⁵ Mette, *Arch. des Sciences biolog.*, 1894, i., 142 (not accessible to me).

⁶ Schütz, *Z. physiol. Ch.*, ix., 577. In *Z. physiol. Ch.*, xxx., 1, he gives a more exact method.

⁷ Schiff, *Lec. de Physiol. de la Digestion*, Berlin, 1867, i., 402.

⁸ Mett, Diss. Petersburg, 1899. Quoted by Roth, *Z. f. klin. Med.*, xxxix., 1, 1900.

⁹ Brücke, *Sitzb. d. kais. Akad. d. Wiss. Wien*, xxxvii., 131.

The digestive power of the gastric juice increases with each rise in the proportion of pepsin it contains (BRÜCKE,¹ MALY,² ELLENBERGER and HOFMEISTER,³ KLUG⁴), but only up to a certain limit (0.1 per cent.). Above that, any excess of pepsin is without effect. CRONER⁵ finds that excessive dilution has a weakening effect in the case of small quantities of pepsin; MAYER⁶ states that although the duration of the digestion is not inversely proportional to the amount of pepsin, it is yet influenced by it. As regards the relation of the temperature to the activity statements vary, but in general 40° C. is regarded as the *optimum*. It is active in *all* acids, but the degree of concentration of these must be different to produce the same results (DAVIDSON and DIETERICH⁷). The most effective acid is said to be *oxalic acid* (WRÓBLEWSKI⁸), but the statements on this point are extraordinarily at variance; almost every investigator arranges them in a different order. In general, it is agreed that *hydrochloric acid* and *lactic acid* are very suitable, and *acetic acid* very unsuitable;⁹ the worst of all is *propionic acid*, according to STUTZER,¹⁰ who, on the other hand, found *malic acid* and *formic acid* to be very effective. *Boric acid* is without influence (HEHNER¹¹). For hydrochloric acid the best concentration is 0.5 to 0.6 per cent. (KLUG⁴).

Certain salts have a restrictive influence on peptic digestion, *e.g.*, common salt¹² and ammonium sulphate (GRÜTZNER,¹³ A. SCHMIDT,¹⁴ KLUG,⁴ MANN;¹⁵ this is also the case with chloroform (BERTELS¹⁶), though only in considerable quantities, whilst small amounts have a stimulating effect (DUBS¹⁷). Similarly,

¹ Brücke, *Vorlesg. über Physiol.*, i., 289.

² Maly in Hermann's *Handbuch d. Physiol.* [5], ii., 83.

³ Ellenberger and Hofmeister, *Arch. f. wiss. u. pract. Thierheilk.*, 1883, ix., 185.

⁴ Klug, *Pflüg. A.*, lx., 52; *ibid.*, lxx., 330.

⁵ Croner, *Virchow's Arch.*, cl., 260.

⁶ Mayer, *Z. f. Biol.*, xvii., 351, 1881.

⁷ Davidson and Dieterich, Müller-Reichert's *Arch. f. Physiol.*, 1860, 690.

⁸ Wróblewski, *Z. f. Physiol. Ch.*, xxi., 1.

⁹ The literature on this point, so far as it is not given here, will be found in Klug, Junr.'s, paper, *Pflüg. A.*, cxv., 330.

¹⁰ Stutzer, *Landwirthsch. Versuchsstat.*, xxxviii., 257, 1891. Cf., however, Nékám (*Maly's Jb.*, xx., 250), who asserts the opposite.

¹¹ Hehner, *Analyst*, xvi., 126, 1891.

¹² This is again denied by Stutzer, *loc. cit.*, 262.

¹³ Grützner, *Pflüg. A.*, xii., 280.

¹⁴ A. Schmidt, *Pflüg. A.*, xiii., 93.

¹⁵ Mann, Diss. Erlangen, 1897.

¹⁶ Bertels, *Virch. A.*, cxxx., 49.

¹⁷ Dubs, *Virch. A.*, cxxxiv., 519.

alcohol in low degrees of concentration (up to 10 per cent.) is without influence, whilst in a 20 per cent. solution all digestion ceases. On the other hand, *beer*, even when containing less than 3 per cent. of alcohol, has a strong restrictive action, and this is *not* to be ascribed to the hops, since *wine* has the same effect (BUCHNER¹). *Carbonic acid*, too, has a retarding influence (SCHIERBECK²), as also has *saccharin* (STUTZER³), and the same is true of infusions of tea and coffee (SCHULTZ-SCHULTZENSTEIN,⁴ FRASER,⁵); but WRÓBLEWSKI⁶ showed that this was only due to the tannin, whereas *caffeine* and *theobromine*, on the contrary, had an *accelerating* action; on the other hand, he found certain alkaloïds to have a strong restrictive effect, notably *veratrine*, *morphine*, and *narceïne hydrochloride*. Moreover, weak solutions of salts, *carbonic acid*, and notably *spices*, such as *pepper*, &c., were found by MANN⁷ to have a beneficial influence, whilst *tobacco juice* had a strong restrictive effect. The presence of small quantities of blood-serum is said to have a retarding influence (GLEY and CAMUS⁸).

CHITTENDEN⁹ has shown, in the case of many substances, that *small quantities* have an accelerating influence, but larger quantities the reverse—*e.g.*, *arsenic* and *arsenious acids*, *chlorides* and *bromides*, *paraldehyde* and *thalline sulphate*. *Antipyrin* and *antifebrin* have a slight restrictive influence, but *quinine*, on the other hand, an accelerating one (WOLBERG¹⁰). *Cane-sugar* in strongly concentrated solutions (40 per cent.) has an injurious action (BUCHNER¹¹). *Potassium sulpho-cyanide* checks the digestion of fibrin by pepsin, but WRÓBLEWSKI¹² attributes this not to any injury to the ferment, but to the shrinking of the fibrin caused by sulpho-cyanide compounds.

An investigation of the influence of a large number of chemical substances as also of sunlight has been made by FERMI and PERNOSI.¹³ All the particulars of their comprehensive research cannot be mentioned here.

¹ Buchner, *Arch. f. klin. Med.*, xxix., 537.

² Schierbeck, *Scand. Arch. f. Phys.*, iii., 357.

³ Stutzer, *Landwirthsch. Versuchsst.*, xxxviii., 1891.

⁴ Schultz-Schultzenstein, *Z. physiol. Ch.*, xviii., 131, 1894.

⁵ Fraser, *Journ. of Anat. and Phys.*, xxxi., 469.

⁶ Wróblewski, *Z. f. physiol. Ch.*, xxi., 1, 1895-6.

⁷ Mann, *Diss.* Erlangen, 1897.

⁸ Gley and Camus, *A. d. Physiolog.*, 1897, 764.

⁹ Chittenden, *Maly's Jb.*, xv., 277; xx., 248.

¹⁰ Wolberg, *Pflüg. Arch.*, xxii., 291, 1880.

¹¹ Buchner, *Ber. d. d. chem. Ges.*, xxx., 1110, 1897.

¹² Wróblewski, *Ber. d. d. chem. Ges.*, xxviii., 1719, 1895.

¹³ Fermi and Pernossi, *Z. f. Hyg.*, xviii., 83, &c.

The fact that pepsin in a neutral solution does not act upon albuminous substances, but only in acid solution (which, too, it was at first assumed, must be a hydrochloric or lactic acid solution), led C. SCHMIDT¹ to the conclusion that pepsin first forms a loose compound with the hydrochloric acid—a *pepsin hydrochloride*—and that this action starts the digestion. But subsequently, when it was possible to show that pepsin acts in *all* acids, it would have been a simple corroboration of Schmidt's theory had the other acids been most active in a state of concentration most suitable for the formation of similar pepsin-acid compounds—i.e., in equi-molecular proportions. That is by no means the case, however, according to DAVIDSON and DIETERICH.² On the contrary no such ratio can be established in the most active degrees of concentration. They themselves suggest another theory in place of Schmidt's³ :—

Only those substances which first cause albuminous bodies to swell up render pepsin capable of decomposing those bodies. Now, all acids do this, but each has for its *optimum* a definite degree of concentration, above and below which its capacity decreases, until finally it becomes *nil*, as does also simultaneously the action of the pepsin. *Ammonia*, however, also acts very energetically upon albuminous matters in causing them to swell up, but destroys pepsin, so that no comparison can be made with it.

The amido-acids of the stomach are also not without influence upon the physiological termination of pepsin digestion owing to their power of combining with hydrochloric acid. HOFFMANN⁴ found that *glycocoll* checks peptic digestion, and ROSENHEIM⁵ asserts the same of *leucine*. SALKOWSKI⁶ has studied this question in detail, and has come to the conclusion that the direct influence of the amido-acids is not considerable.

The final extinction, however, of the action of pepsin appears to be closely connected with this question.

Although the accumulated *decomposition products*—e.g., albumoses, &c.—appear to have a direct influence upon pepsin, the primary cause is the disappearance of the *hydrochloric acid*. As GÜRBER⁷ demonstrated, digestion which has ceased can again be started by adding to the mixture a few drops of hydrochloric acid. On the cessation of the action of pepsin free hydrochloric

¹ C. Schmidt, *Liebig's Ann.*, lxi., 311.

² Davidson and Dieterich, *Arch. f. Anat. u. Physiol.*, 1860, 690.

³ *Loc. cit.*, 701. ⁴ Hoffmann, *Centralbl. f. klin. Med.*, 1891, 793.

⁵ Rosenheim, *ibid.*, 1891, 729.

⁶ Salkowski, *Virch. A.*, cxxvii., 501; *med. Wiss.*, 1891, 945.

⁷ Gürber, *Verh. Würzb. phys. med. Ges.*, 1895, 67.

acid (which can be detected by Günzburg's reagent or its inverting power) is, in fact, *no longer present*. Gürber assumes that it has entered into combination with the decomposition products, 5 atoms of albuminous nitrogen combining, in his opinion, with 1 molecule of hydrochloric acid. Although this speculation appears somewhat daring, and though the other quantitative reasons—viz., that *deuteroalbumoses* combine with more hydrochloric acid than *protoalbumoses*, but *peptones*, again, with less, are necessarily unconvincing, yet there appears to be no doubt as to the fact of the combination of hydrochloric acid with albuminoid substances, which Gürber considers the *amido-acids* to be (thus not drawing an *analogy* which alone would have been justifiable). Indirectly, then, the accumulated decomposition products influence, at all events, the fermentation, if not the ferment.

The substances which are attacked by pepsin are *the whole of the genuine albuminous substances*, dissolved and coagulated alike,¹ and the *nucleo-albumins* (e.g., *casein*); also *collagene*, *glutin*, *chondrogen*, *chondrin*, *elastin*, and *oxyhæmoglobin*, but not *mucin*, *keratin*, or *chitin*. Further particulars, especially of the decomposition products, I must reserve for a later part of this book.

The rapidity with which the individual albuminous bodies are dissolved and digested by pepsin, and the nature of the resulting products, vary with the particular proteids and different pepsins. Casein² is said to be dissolved the most easily. The value of these investigations is very small, owing to the invariable discordance in the results and the differences, both in the experimental conditions and the methods of identification.

When introduced into the circulation, it deprives the blood of its power of coagulating (ALBERTONI³), as is also done by other enzymes.

CHARRIN⁴ regards the power possessed by pepsin of rendering bacterial toxins innocuous as a very important function for the protection of the organism. It is, however, doubtful how far this action must not be attributed to the acid of the stomach alone; for, according to Fermi and Pernossi,⁵ tetanotoxine is *not* changed by pepsin. Although, according to GAMALEÏA,⁶ *diphtheria toxine* is decomposed with the formation of a nucleïn, its poisonous property is *not* destroyed, for the action of the poisonous nucleïn, *in vivo*, is said to be conditional on this decomposition.

¹ Fick, *Verh. d. Würzb. phys. med. Ges.*, 1872, 113.

² See, *inter alios*, Klug, jun., *Pflüg. A.*, lxx., 330, 1897.

³ Albertoni, *C. f. d. med. Wiss.*, 1878, 3.

⁴ Charrin, *Archiv. de Physiol.*, Fifth Series, x., 65.

⁵ Fermi and Pernossi, *Z. f. Hyg.*, xvi., 385, 1894.

⁶ Gamaleïa, *C. R. Soc. Biol.*, xlv., 153, 1892.

Other investigators, too, ascribe to the gall the main activity in the destruction in the digestive tract of these poisons which have found their way, *per os*, into the organism (FRASER and others). A *peptotoxine* was found by BRIEGER¹ in the digestion of fibrin, but its existence is denied by SALKOWSKI,² who attributes the poisonous action to the peptones.

The Products formed in Digestions with Pepsin.—The process of peptic decomposition is, without doubt, a breaking-up of groups of higher molecules into lower ones. Eventually there are formed, from the absolutely indiffusible albuminous bodies, diffusible substances—*peptones*. That this decomposition is, in the main, a *hydrolytic* process may be inferred from the fact that the products formed in peptic digestion are analogous to those which result on boiling albuminous substances with dilute acids and bases, or even with superheated water (steam).

To follow this process in its details, chemically, is at present impossible, since the constitution not only of the albuminous bodies themselves, but also of their peptic decomposition-products, are quite unknown to us. We must be content, with the aid of certain precipitation and colour reactions, to make a general survey of the different phases of the process, which, as was mentioned, effects a gradual breaking-up of non-diffusible *albuminous substances* into diffusible *peptones*. Between these, certain intermediate products, which are still but little diffusible but yet different from the *albuminous substances*, have been discovered, and to these the name of *albumoses* has been given. We should far exceed the scope of this book if we were to follow in detail the exceedingly numerous and laborious investigations which have brought us to our present standpoint; we are only able to depict the decomposition of albuminous substances according to modern views in its main outlines, selecting the essential points.

The first discoveries in this field were made by MEISSNER³ and his pupils. He described several *peptones* which partly corresponded to our *albumoses* of to-day, partly to the so-called "true" peptones, and partly to Kühne's anti-albuminate. Further fundamental researches were published by SCHÜTZENBERGER.⁴

The modern views are based, in the main, on the classical researches of KÜHNE and his pupils. Kühne found that, in

¹ Brieger, *Z. physiol. Ch.*, vii., 274; *Ueber Ptomaine*, 1885, 14, &c.

² Salkowski, *Virch. A.*, cxxiv., 409.

³ Meissner, *vide Zeitschr. f. rat. Medic.* (3), vii., 1; viii., 280; xi.; xiv., 303.

⁴ Schützenberger, *Bull. de la Soc. Chim. de Paris*, xxiii., 1, 161, 193, 216, 242, 385, 433; xxiv., 2, 145.

pancreatic digestion (*q.v.*), approximately half of the albuminous material escaped, in the form of a peptone, the more thorough decomposition which the other half underwent. Exactly the same result was produced when he submitted to the decomposing action of trypsin the total quantity of peptones which he obtained in the gastric digestion of albuminous substances, and to which he gave the name of *ampho-peptones*. Whilst the one-half, the so-called *hemi-peptone*, was decomposed in the characteristic manner by trypsin, the other half, the *anti-peptone*, remained unaffected by these influences.

Kühne concluded from this that there were two distinct groups in the albumin molecule, the *hemi-* and the *anti-group*, and that these were separately decomposed.

Then from the anti-group *anti-albuminate* (Meissner's para-peptone) is formed when the amount of pepsin is *insufficient*.

This is soluble in acids and precipitated by alkalies. It can also be obtained by boiling albumin with dilute acids; as thus prepared it is not attacked by pepsin, but is converted by trypsin into *anti-peptone*.

When the pepsin is in large excess, however, *no* anti-albuminate is produced, but first *anti-* and *hemi-albumoses*, and then *anti-* and *hemi-peptones*. The hemi-albumoses, which KÜHNE¹ first isolated, proved to be a mixture of different compounds, including anti-albumoses.

Subsequently, KÜHNE and CHITTENDEN² succeeded in separating the different albumoses produced in peptic decomposition.

The liquid from the digestion is neutralised with sodium hydroxide solution, and the resulting precipitate triturated with common salt. On now treating it with a concentrated solution of salt, part of it, termed *deutero-albumose*, is *dissolved*. This is precipitated from the salt solution by means of acetic acid.

The residue is treated with water. With the exception of a trifling residue, *dysalbumose*, it is completely dissolved. On dialysing the solution the *proto-albumoses* remain in solution, while the *hetero-albumoses* are precipitated. For the systematic classification of these different albumoses *vide infra*:—

Proto-albumose is soluble in water, and is not coagulated on boiling. It is insoluble in a concentrated solution of common salt. *Nitric acid* gives a precipitate soluble in excess; it forms a precipitate with acetic acid and potassium ferrocyanide, as also with copper sulphate, mercuric chloride, and lead acetate. It is also soluble in alcohol, and can thus be separated from the hetero-albumoses (PICK).³

¹ Kühne, *Z. f. Biol.*, xix., 209.

² Kühne and Chittenden, *Z. f. Biol.*, xix., 153; xx., 11.

³ Pick, *Z. f. physiol. Ch.*, xxviii., 219, 1899.

Hetero-albumose is insoluble in water, but soluble in dilute solutions of salt, in acids, and in alkalies. When suspended in water it coagulates on boiling, and becomes insoluble in solutions of salt. The salts of heavy metals precipitate it. Particularly interesting is the discovery of SPIRO,¹ that hetero-albumoses on further decomposition yield principally *leucine*, and also *glycocoll*, whereas the proto-albumoses, on the other hand, yield much *tyrosin* but no *glycocoll*—a fact which points to a deep-seated difference between them.

Deutero-albumose is soluble in a saturated solution of common salt, and in distilled water. It is not precipitated by a 2.5 per cent. solution of copper sulphate, or by nitric acid.

Dysalbumose is regarded as a modification of hetero-albumose, which is soluble in a dilute solution of salt.

The general reactions of albumoses are:—

They are precipitated by ammonium sulphate.²

Nitric acid gives a precipitate, which dissolves on heating, as is also the case with acetic acid and potassium ferrocyanide, but small differences are here perceptible.

Copper sulphate with sodium hydroxide gives red to reddish-violet colorations, which are not quite uniform in tint, as they depend on the mode of applying the test (*Biuret reaction*).

They are lævorotatory.

Analogous albumoses have been prepared from other *albuminous bodies*:—*Globuloses* from *globulin* (KÜHNE and CHITTENDEN,³ CHITTENDEN and HARTWELL⁴), *vitelloses* from *vitellin* (NEUMEISTER,⁵ CHITTENDEN and MENDEL⁶), and *myosinoses* (KÜHNE and CHITTENDEN,⁷ CHITTENDEN and GOODWIN⁸), which in like manner can be further designated proto-, deutero-, &c. As regards the decomposition of albuminoid substances *vide infra*.

NEUMEISTER⁹ has based a theory of the decomposition of albumin by pepsin, on Kühne's results and conclusions as well as on his own researches. He assumes that there are two main varieties of albumoses, those which are still closely related to the albuminous bodies—*primary* albumoses—and those which are more closely related to the peptones—*secondary* albumoses. Both groups are derived from both the *hemi*- and the *anti*-group of the albumin molecule, although the *proto-albumoses* are principally derived from the *hemi-group* and the *hetero-albumoses* principally from the *anti-group*; not that, however, the reciprocal groups are entirely unrepresented. Both of them

¹ Spiro, *Z. f. physiol. Ch.*, xxviii., 174, 1899.

² Wenz, *Z. f. Biol.*, xxii., 10, 1886.

³ Kühne and Chittenden, *Z. f. Biol.*, xxii., 409.

⁴ Chittenden and Hartwell, *Journ. of Physiol.*, xi., 435, 1890.

⁵ Neumeister, *Z. f. Biol.*, xxiii., 381.

⁶ Chittenden and Mendel, *Journ. of Physiol.*, xvii., 48, 1895.

⁷ Kühne and Chittenden, *Z. f. Biol.*, xxv., 358.

⁸ Chittenden and Goodwin, *Journ. of Physiol.*, xii., 34.

⁹ Neumeister, *vide inter alia*, *Lehrb. d. physiol. Ch.*, 1893, 1.

yield secondary albumoses, which are grouped together under the name of *deutero-albumose* (*ampho-albumose*), and then *ampho-peptone*, which naturally in the case of the hetero-albumose must contain more anti-peptone, and in the case of the proto-albumose more hemi-peptone.

The views of Kühne and Neumeister have not been left without contradiction. HAMBURGER¹ will not admit the distinction between the albumoses, but ascribes the varying ratios of solubility, &c., to different mixture-coefficients which are able to bring about these phenomena in *one* albumose. MOROCHOWETZ,² too, is opposed to Kühne's views.

The whole theory of the albumoses, and their relations to the albumin molecule in general, has quite recently experienced numerous modifications and contradictions.

E. P. PICK³ succeeded in obtaining by fractional saturation with ammonium sulphate no less than three deutero-albumoses, A, B, and C, and *two* peptones from Witte's peptone, and his observations have been found by UMBER⁴ and ALEXANDER⁵ to also apply to pure albuminous bodies. E. ZUNZ,⁶ who has quantitatively examined the decomposition of several pure albuminous substances with the aid of BAUMANN and BÖMER's⁷ method of precipitation with zinc sulphate, is also greatly inclined to doubt the individual nature of the deutero-albumoses, A and B, so that in that case *five* deutero-albumoses would exist. But besides this he has proved that deutero-albumose A, and above all B, are formed at as early a stage as the so-called "primary" albumoses, and so have as much right to be designated by that title. Moreover, substances of a still unknown nature, which *no longer give the biuret reaction* (*vide infra*) are primarily formed. Kühne's whole scheme is thus very open to attack. To this must be added the fact that, according to ALEXANDER,⁵ casein forms no proto-albumose, and yet is decomposed in pancreatic digestion. Moreover, KÜHNE⁸ himself has found that the deutero-albumoses diffuse with more difficulty than the proto-albumoses, which also supports the view that they are more closely akin to the true albuminous substances in character.

¹ Hamburger, *Maly's Jb.*, xvi., 20.

² Morochowetz, *St. Petersburg med. Woch.*, 1886. No. 15.

³ Pick, *Z. f. physiol. Ch.*, xxix., 246.

⁴ Umber, *ibid.*, xxv., 258.

⁵ Alexander, *ibid.*, xxv., 411.

⁶ E. Zunz, *Z. physiol. Ch.*, xxvii., 219; xxviii., 132.

⁷ Baumann and Bömer, *Z. f. Untersuch. v. Nahr.- u. Genussm.*, i., 106, 1898.

⁸ Kühne, *Z. f. Biol.*, xxix., 20, 1893.

Peptones.—According to the views of Kühne and his pupils these are the final products of peptic digestion. They are distinguished from the albumoses by not being precipitated from an acid solution by ammonium sulphate (WENZ¹). They diffuse much more readily through animal membranes, and have a different percentage composition, notably a lower proportion of carbon. As was mentioned above they were discovered by Meissner, who also gave them their name.

Methods of preparing them have been proposed by HENNINGER² and HERTH,³ but they do not yield peptones free from albumose. KÜHNE⁴ has described several methods of obtaining pure peptones, which have subsequently been more fully worked out by PICK, ZUNZ,⁵ and others.

Peptones are not precipitated either by boiling, by acids, or by acetic acid with potassium ferrocyanide.

They are precipitated by the salts of several heavy metals, by tannin, and, above all, by phospho-tungstic acid, which, in particular, is a favourite method of separating them. They give Millon's reaction and the characteristic *red* biuret reaction with a dilute solution of sodium hydroxide and copper sulphate. They are lævorotatory.

In a pure state they have *not* a restricted influence on coagulation (POLLITZER⁶) as had previously been found to be the case with preparations containing albumose (SCHMIDT-MÜLHEIM,⁷ FANO,⁸) and they differ directly from the albumoses in this respect.

Kühne and his pupils have assumed, as a consequence of their fundamental conception, the existence of a *hemi-peptone* and an *anti-peptone*. The latter is stated to be an individual substance incapable of being further changed by trypsin.

It does *not*, like hemi-peptone, give a red coloration with Millon's reagent (KÜHNE and CHITTENDEN).

By the recent researches of KUTSCHER,⁹ however, it has almost been proved that Kühne's *anti-peptone* does not exist as such, at least not in the quantity assumed (half of the total products), but is a mixture of different substances formed in the

¹ Wenz, *Zeitschr. f. Biol.*, xxii., 10.

² Henninger, *Comptes Rendus*, lxxxvi., 1413, 1464.

³ Herth, *Z. f. physiol. Ch.*, i., 279.

⁴ Kühne and Chittenden, *Z. f. Biol.*, xxii., 425. Kühne, *ibid.*, xxix., 1.

⁵ *Loc. cit.*

⁶ Pollitzer, *Journ. of Physiol.*, vii., 283. Cf. *Verh. naturh. med. Ver. v. Heidelberg*. New Series, iii., 293.

⁷ Schmidt-Mülheim, *Du Bois A. f. Phys.*, 1880, 50.

⁸ Fano, *ibid.*, 1881, 277.

⁹ Kutscher and others, *Die Endproducte der Tripsinverdauung*, 1899.

tryptic decomposition. By these discoveries the whole conception of the hemi- and anti-group in the albumin molecule is fundamentally shaken.

Other Products of Peptic Decomposition.—Some time ago HOPPE-SEYLER¹ stated that other substances, probably of a more simple nature like that of the amido-acids, were formed in the action of pepsin. PFAUNDLER² claims to be able to exclude amido-acids. CHITTENDEN and HARTWELL³ were unable to accomplish a complete conversion of albuminous substances into peptones. Moreover, the results obtained by LAWROW⁴ support the conclusion that there are further decomposition products, as do also, in particular, the researches of E. ZUNZ.⁵ The latter found that when the action of pepsin was very energetic relatively little peptone was formed, but considerable quantities of nitrogenous substances, which were unprecipitable by phospho-tungstic acid, though precipitated by tannin, and gave *no* biuret reaction. They occurred at an early stage of the decomposition, and thus belonged to the "primary" decomposition products. Their nature is still unknown.

Action of Pepsin on Albuminoid Substances.—*Collagene* and *glutin* are decomposed by pepsin into *gelatose* and *glutin peptone*, the collagene being first converted into glutin (ETZINGER,⁶ UFFELMANN,⁷ TARTARINOFF,⁸ KLUG,⁹ v. GERLACH.¹⁰)

Chondrogen and *chondrin* are decomposed more slowly, but in a similar manner. In addition to pepsin a reducing substance is formed in the process.¹¹

Mucin, according to KÜHNE and SCHIFF, is not attacked by pepsin.¹²

Elastin yields bodies of an albumose nature (ETZINGER,⁶ GAMGEE, HORBACZEWSKI,¹³ MOROCHOWETZ,¹⁴ CHITTENDEN and HART).¹⁵ *Keratin*, *chitin*, *conchiolin*, and *spongine* are not attacked.¹¹

¹ Hoppe-Seyler, *Physiolog. Chemie*, 1881, ii., 228.

² Pfaundler, *Z. f. physiol. Ch.*, 1900, xxx., 90.

³ Chittenden and Hartwell, *Journ. of Physiol.*, xii., 12, 1891.

⁴ Lawrow, *Z. f. physiol. Ch.*, xxvi., 513, 1898.

⁵ E. Zunz, *Z. f. physiol. Ch.*, xxviii., 172, 1899.

⁶ Etzinger, *Z. f. physiol. Ch.*, xxvi., 513, 1898.

⁷ Uffelmann, *A. f. klin. Med.*, xx., 535.

⁸ Tartarinoff, *Centralbl. f. med. Wiss.*, 1877, 275.

⁹ Klug, *Centralbl. f. Physiol.*, iv., 189.

¹⁰ v. Gerlach, *Die Peptone*, 1891. ¹¹ Hoppe-Seyler, *Physiol. Ch.*, 234.

¹² Gamgee, *Phys. Ch. d. Verdauung*, 159, 160.

¹³ Horbaczewski, *Z. physiol. Ch.*, vi., 330.

¹⁴ Morochowetz, *Maly's Jb.*, 1886, 271.

¹⁵ Chittenden and Hart, *Z. f. Biol.*, xxv., 368, 1889.

Oxyhæmoglobin is decomposed into albumoses and peptones, hæmatin being split off.¹

From *casein* several "propeptones" and a peptone were first obtained by THIERFELDER.²

Casein-albumoses, *caseoses*, were then described by CHITTENDEN and PAINTER.³

Next, SEBELIEN⁴ obtained albumoses and (optically inactive [?]) peptones from casein, as was also done by SALKOWSKI.⁵ Finally ALEXANDER⁶ has again investigated the subject with the aid of Pick's method (*vide supra*), and has prepared substances corresponding to Pick's albumoses. *Hetero-albumose* was almost entirely absent.

In addition to the typical decomposition-products of albumin, substances containing phosphorus are naturally obtained from casein, and partially pass into solution (LUBAVIN,⁷ SALKOWSKI and HAHN,⁸ v. MORACZEWSKI,⁹ who found varying amounts of pseudonuclein, and others). Bodies resembling the nucleins have also been found in the peptic digestion of casein by CL. WILLDENOW¹⁰ and WRÓBLEWSKI,¹¹ the latter obtaining the nuclein only from the casein of cow's milk, and not from that of human milk. *Nuclein* is not attacked by pepsin (MIESCHER¹²).

The peptic digestion of the *gluten-casein* of wheat has been investigated by CHITTENDEN and SMITH.¹³

Chlorophyll is partially digested by pepsin in hydrochloric acid, the *metaxin* being attacked, but the *chloroplastin*, on the other hand, not changed (SCHWARZ¹⁴). *Pepsin* has frequently been proved to have a digestive influence on *other ferments*.

¹ Hoppe-Seyler, *Physiol. Ch.*, 233.

² Thierfelder, *Z. physiol. Ch.*, x., 577.

³ Chittenden and Painter, *Studies from the Laboratory of Physiol. Chem. of Yale College*, ii., 1885-86, 156 (*Maly's Jb.*, xvii., 16; xx., 17).

⁴ Sebelien, *Chem. Centralbl.*, 1890, i., 170.

⁵ Salkowski, *Centralbl. f. med. Wiss.*, 1893, 385.

⁶ Alexander, *Z. f. physiol. Ch.*, xxv., 411.

⁷ Lubavin, Hoppe-Seyler's *Untersuch. z. med. Chemie.*, i., 463.

⁸ Salkowski and Hahn, *Pflüg. A.*, lix., 225.

⁹ v. Moraczewski, *Z. f. physiol. Ch.*, xx., 28 (Comprehensive bibliography).

¹⁰ Clara Willdenow, *Z. Kenntn. d. pept. Verd. d. Caseins*, Diss. Berne, 1893.

¹¹ Wróblewski, *Beiträge zur Kenntniss des Frauencaseins und seiner Unterschiede vom Kuhcasein*, Diss. Berne, 1894.

¹² Miescher quoted by Bokay, *Z. physiol. Ch.*, i., 157.

¹³ Chittenden and Smith, *Journ. of Phys.*, xi., 410.

¹⁴ Schwarz, quoted by Cohn, *Beitr. z. Biol. d. Pflanzen*, v., 73.

CHAPTER X.

TRYPSIN.

Trypsin, which received its name from Kühne, is the proteolytic ferment of the *pancreas*.

Like pepsin, it is an unorganised ferment, so that its action can be studied not only in the organ itself, but also in its secretions and in the preparations obtained from it.

The action of the pancreatic juice upon albuminous substances was, as CORVISART¹ states, observed as early as the year 1836 by PURKINJE and PAPPENHEIM. It was also recognised, but not estimated at its proper value, by CLAUDE BERNARD.² This important phenomenon was more closely studied, first by CORVISART and then by MEISSNER.³ Then came the researches of KÜHNE and his pupils, notably DANILEWSKY.⁴ It was shown by KÜHNE⁵ that, through the agency of the pancreatic tissue and the tissue of the glands, albuminous bodies underwent a decomposition which was distinguished from that due to pepsin by the fact that *leucine* and *tyrosine* were formed in addition to peptones. The process of the secretion was first studied by HEIDENHAIN.⁶ Notwithstanding the fact that the pronounced effect of the ferment of the pancreas on albuminous bodies may be readily proved, the pancreas is nevertheless not an organ absolutely essential to life. It has, indeed, been stated that atrophy of the pancreas (*e.g.*, after binding up the outlet) causes absolutely no metabolic anomalies. ROSENBERG⁷ has shown, however, that the want of pancreatic juice in the intestine is accompanied by a diminution in the transformation of albumin.

¹ Corvisart, *Sur une fonction peu connue du Pancréas*, Paris, 1857-8. Cf. *Z. f. ration. Med.* (3), vii., 119.

² Cl. Bernard, *Lec. d. physiol. expér.*, ii., 1856.

³ Meissner, *Zeitschr. f. rat. Med.* (3), vii., 1859.

⁴ Danilewsky, *Virch. A.*, xxv., 1862, 267.

⁵ Kühne, *Virch. A.*, xxv., 1862, 267 (gives all the older literature).

⁶ Heidenhain, *Pflüg. A.*, x., 557.

⁷ Rosenberg, *Pflüg. A.*, lxx., 371 (gives the literature on the subject).

Preparation of Trypsin.—When alcohol is added to the pancreatic juice, a precipitate containing trypsin is formed, to which the name of *pancreatin* was given. It still contains, however, albuminous substances, which KÜHNE¹ grouped together under the name *leucoid*, and other impurities which he was able to eliminate to a large extent by dissolving it in water at 0° C., precipitating it in successive fractions with acetic acid and sodium hydroxide, and finally using dialysis. A more suitable method was afterwards given by Kühne. HAMMARSTÉN² extracts the pancreas with ammonium hydroxide of 0.03 per cent. strength and adds acetic acid. The precipitate is then dissolved in a solution of sodium hydroxide. GULEWITSCH³ uses for the extraction a solution of sodium hydroxide containing chloroform and thymol, whilst LOEW⁴ employs 40 per cent. alcohol, and precipitates with a mixture of alcohol and ether.

Active solutions of the ferment, in which, however, the other ferments of the pancreas are present, have been obtained by extraction with glycerin (HEIDENHAIN,⁵ v. WITTICH⁶), with chloroform water, boric acid solution, and sodium chloride solution (ROBERTS,⁷ HARRIS and Gow⁸), and with salicylic acid solution (KÜHNE⁹). PASCHUTIN¹⁰ has made experiments on the extraction of the different ferments of the pancreas with solutions of salts. Solutions of potassium iodide, sodium arsenite, sodium sulphite, oxalic acid, and tartaric acid are among the best solvents for *trypsin*, and are thus suitable for the preparation of active digestive solutions.

Properties of Trypsin.—As is the case with all the ferments, it has not been obtained in a pure condition. Like diastase, *trypsin* appears to have a very complex constitution, akin to that of the albuminous bodies; in Kühne's opinion, its composition is even more complex than theirs, since, on heating it with dilute acids, albuminous substances are first split off in the decomposition. It is easily soluble in water and in mixtures of glycerin and water. In pure glycerin and concentrated alcohol

¹ Kühne, *Verh. naturh. med. Ver. Heidelberg.*, New Series, i. (1876), 194; and iii., 463.

² Hammarstén, *Lehrb. phys. Ch.*, 1895, 265.

³ Gulewitsch, *Z. physiol. Ch.*, xxvii., 544, 1899.

⁴ Loew, *Pflüg. A.*, xxvii., 207, 1882.

⁵ Heidenhain, *Pflüg. A.*, x., 557.

⁶ v. Wittich, *Pflüg. A.*, ii., 196.

⁷ Roberts, *On the Digestive Ferment*, Lumleian Lecture, 1880, London, 26. Quoted by Gamgee, *loc. cit.*

⁸ Harris and Gow, *Journ. of Physiol.*, xiii., 469.

⁹ Kühne, *Unters. physiol. Inst. Heidelberg*, i., 222, 1878.

¹⁰ Paschutin, Müller-Reichert's *A. f. Phys.*, 1873, 382.

it is insoluble, but soluble, on the other hand, in alcohol of 40 per cent. strength (DASTRE¹).

It works best in a weak alkaline solution (1 per cent. sodium hydroxide); also, according to SCHIERBECK,² in neutral and faintly acid solutions, but best in very dilute acid solutions. Its action would thus approximate more closely to that of the other enzymes, which also have their *optimum* in weak acid solutions. According to KÜHNE,³ it remains active until the percentage of acid reaches 0.05 per cent., and this has been confirmed by LANGLEY;⁴ on the other hand, EWALD⁵ asserts that it is still active with 0.3 per cent. of acid. More concentrated acids naturally have a very pronounced injurious effect; this influence, is paralysed, or there may even be a certain degree of stimulation when the acids are combined with albuminous bodies (CHITTENDEN and CUMMINS⁶).

Gall favours the action of trypsin specially in the presence of *lactic acid* (LINDBERGER⁷), and also of hydrochloric acid (RASCHFORD and SOUTHGATE⁸).

Like all ferments it is rapidly destroyed by more concentrated alkalies. *Carbonic acid* has a stimulating influence in a liquid of alkaline reaction, since it diminishes the alkalinity, but has a restrictive effect in an acid solution (SCHIERBECK²).

The action of neutral salts has frequently been studied, but first systematically by PODOLINSKI.⁹ He found that *all* salts promote the action of trypsin, but that the intensity of this influence greatly varies. Sodium salts have the most pronounced effect.

On the other hand, neutral *ammonium phosphate* has been shown to have rather a restrictive influence, as have also, *inter alia*, *mercuric* and *iron salts* (CHITTENDEN and CUMMINS⁶).

CHITTENDEN and STEWART¹⁰ investigated the effect of drugs, and found that in small doses they frequently had a stimulating action, but a restrictive one in larger amounts, though in general to a slighter extent on trypsin than on pepsin. *Paraldehyde*, in particular, had a most injurious influence.

¹ Dastre, *Arch. d. Physiol.*, 1896, 120.

² Schierbeck, *Scand. Arch. f. Physiol.*, iii., 344.

³ Kühne, *Verh. naturh. med. Ver. Heidelberg*, New Series, i.

⁴ Langley, *Journ. of Phys.*, iii., 263. ⁵ Ewald, *Z. klin. Med.*, i., 615.

⁶ Chittenden and Cummins, *Maly's Jb.*, 1885.

⁷ Lindberger, *Maly's Jb.*, xiii., 280, 1883.

⁸ Raschford and Southgate, *Medical Record*, xii., 95; *Maly's Jb.*, xxvi., 392, 1896.

⁹ Podolinski, *Beitr. z. Kenntn. d. pancr. Eiweissverd.*, Diss. Breslau, 1876.

¹⁰ Chittenden and Stewart, *Maly's Jb.*, xx., 248, 1890.

A comprehensive research on the influence of all kinds of substances on trypsin was published by FERMI and PERNOSI,¹ to which we can only allude here.

It is rapidly rendered inactive by the action of pepsin in acid solution (MAYS,² LANGLEY³). In KÜHNE'S⁴ opinion this is physiologically important, as affording an explanation of the significance of the gall in the digestive process. The gall normally precipitates the pepsin; but when this function is absent—*e.g.*, in fistula—the pepsin penetrates into the intestine and destroys the trypsin to a more or less pronounced extent, and thereby the digestion of the albumin is checked.

Its activity increases up to about 60° C., then falls rapidly, and ceases at 75° to 80° C. (ROBERTS⁵).

On the other hand, BIERNACKI⁶ states that in a faintly alkaline solution it becomes inactive at as low a temperature as 50° C., and in a neutral solution at 45° C. Similarly HEIDENHAIN⁷ asserts that it loses its activity when maintained for a long time at a temperature of 35° C.—a statement which is opposed by KÜHNE,⁸ SALKOWSKI,⁹ and EWALD.¹⁰

In the dry condition it resists the action of heat (HÜFNER¹¹) up to 160° C. (SALKOWSKI¹²). When heated in *ether* in a sealed glass tube it is destroyed at as low a temperature as 80° C., but in *amyl alcohol* it is still active at 100° C. (FERMI and PERNOSI).¹

Trypsin has been found in the foetus by ALBERTONI¹³ and by FERMI¹⁴ *inter alios*.

Outside the pancreas it has been found by SAHLI,¹⁵ GEHRIG,¹⁶ TASULLI,¹⁷ DASTRE and FLORESCO,¹⁸ and BENDERSKY,¹⁹ in the urine, where, however, LEO,²⁰ and STADELMANN²¹ were unable to detect

¹ Fermi and Pernossi, *Z. f. Hyg.*, xviii., 83.

² Mays, *Unters. physiol. Instit. Heidelb.*, iii., 378.

³ Langley, *Journ. of Physiol.*, iii., 263.

⁴ Kühne, *Verh. naturh. med. Ver. Heidelberg*, 1877, 190.

⁵ Roberts quoted by Gamgee, *loc. cit.*, 235.

⁶ Biernacki, *Z. f. Biol.*, xxviii., 62. ⁷ Heidenhain, *Pflüg. A.*, x., 557.

⁸ Kühne, *Verh. naturh. med. Ver. Heidelberg*, New Series, i., 196, 1876.

⁹ Salkowski, *Virch. A.*, lxx., 158.

¹⁰ Ewald, *Lehre von d. Verdauung*, 1878, 8.

¹¹ Hüfner, *J. pr. Ch.*, New Series, v., 372.

¹² Salkowski, *Med. Centralbl.*, 1876, 29; *Virch. A.*, lxx., 158.

¹³ Albertoni, *Maly's Jb.*, viii., 254, 1878.

¹⁴ Fermi, *Maly's Jb.*, 1892, 592.

¹⁵ Sahli, *Pflüg. A.*, xxxvi., 224. ¹⁶ Gehrig, *Pflüg. A.*, xxxviii. 35.

¹⁷ Tasulli, *Maly's Jb.*, 1894, 289.

¹⁸ Dastre and Floresco, *C. R. Soc. Biol.*, 1897, 849.

¹⁹ Bendersky, *Virch. A.*, cxxi., 554.

²⁰ Leo, *Pflüg. A.*, xxxvii., 226; xxxix., 246.

²¹ Stadelmann, *Z. f. Biol.*, xxiv., 226.

it. KUHNE¹ found it nowhere except in the pancreas and contents of the intestine. The *histozyme* obtained from the kidneys by Schmiedeberg is in NENCKI'S² opinion probably a pancreatic ferment. HOFFMANN³ only found trypsin in the urine when the pancreatic juice was excluded from the intestine, but never under other circumstances; he found it, however, in the *spleen* and other organs. FERMI and PERNOSI⁴ were able to detect it in the urine after subcutaneous injection. It was found in the sputum of those suffering from diseases of the lungs by FILEHNE⁵ and ESCHERICH,⁶ notably in bronchiectasis and phthisis.

As regard other vertebrate animals, it has been found to an equal extent in those which possessed a pancreatic gland.

KRUKENBERG⁷ discovered it in the stomach and intestine of many *fishes*; HOMBURGER⁸ found proteolytic ferments of similar character in the *cyprinoidæ*; HOPPE-SEYLER⁹ discovered a trypsin in *cray-fish*, and BIEDERMANN¹⁰ in *meal-worms*. A very interesting observation, which points to the presence of trypsin in the egg, was made by GAYON.¹¹ He discovered *tyrosine* in *non-putrid* eggs. The contents of the egg were fluid; micro-organisms were not present. Subsequently MROCZKOWSKI¹² found a tryptic ferment in the dried *albumin of hen's egg*.

The action of trypsin can be detected and an approximate estimation made by means of fibrin, which, as suggested by GRÜTZNER (see under *Pepsin*), has been coloured with Magdala red. It is placed upon a filter and the coloured drops which fall from the filter, when the trypsin is active, are noted (and eventually counted) (GEHRIG, *loc. cit.*). ROBERTS¹³ makes use of the occurrence or disappearance of the metacasein reaction (*vide infra*) for the estimation of the proteolytic power.

Different animals vary as regard the energy of their pancreatic digestion. In the case of the proteolytic ferments, FLORESCO¹⁴ found they could be arranged in the following order

¹ Kühne, *Verh. naturh. med. Ver. Heidelberg*, 1880, 1.

² Nencki, *A. exp. Path.*, xx., 376.

³ Hoffmann, *Pflüg. A.*, xli., 148, 1887.

⁴ Fermi and Pernossi, *Z. f. Hyg.*, xviii., 125.

⁵ Filehne, *Sitzb. d. Erlanger phys. med. Soc.*, 1877, 169.

⁶ Escherich, *Arch. f. klin. Med.*, xxxvii., 196, 1885.

⁷ Krukenberg, *Unters. physiol. Inst. Heidelberg*, 1882, ii., 396.

⁸ Homburger, *C. med. Wiss.*, 1877, 561.

⁹ Hoppe-Seyler, *Pflüg. A.*, xiv., 394.

¹⁰ Biedermann, *Pflüg. A.*, lxxii., 160.

¹¹ Gayon, Thèse. Paris, 1875, quoted by Schützenberger, *loc. cit.*, 199.

¹² Mroczkowski, *Biolog. Centralbl.*, ix., 154, 1889-90.

¹³ Roberts, *Proceed. Royal Society*, xxxii., 145.

¹⁴ Floresco, *Comptes Rend. d. l. Soc. d. Biol.*, 1896, xlvi., 77, 890.

in this respect: pig, dog, ox, sheep; gelatin, however, was acted upon most energetically by the ferment of the dog, and then by those of the pig, sheep, and ox. When introduced into the circulation *trypsin* prevents the coagulation of the blood and destroys *leucocytes* (ALBERTONI).¹

The juice of the pancreas is said to have a special action upon milk or casein solution, which it causes to coagulate though in a different way to rennet (*metacasein reaction*) (KÜHNE,² HALLIBURTON and BRODIE³). It causes, *inter alia*, the casein thus altered to coagulate on boiling, to give a precipitate with sodium chloride, &c. (EDKINS⁴). According to ROBERTS⁵ this *metacasein* represents the first tryptic digestive product of casein.

The Zymogen of Trypsin.—It was discovered by HEIDENHAIN⁶ that the pancreas did not contain ready-formed trypsin, but only its antecedent, which on suitable treatment decomposed with the formation of the actual enzyme.

PODOLINSKY⁷ then investigated this zymogen more fully. It is separated from the gland by means of a neutral solution of glycerin, in which it is stable. From this solution it can be precipitated by means of alcohol, and dissolved in sodium hydroxide solution without being transformed into the ferment. This, however, is denied by Kühne.

It passes into the active form on exposure to the air, diluting the glycerin solution with water, and on treatment with acids. Podolinsky assumes that in this change oxygen plays a part, since hydrogen peroxide and platinum black also produce a fermentative reaction. He endeavoured to recover the zymogen from the ferment by means of reduction. Although phosphorus and zinc dust had no effect, the action of the enzyme was weakened by yeast, and again strengthened by the introduction of oxygen. All salts have an influence on the decomposition.

According to HERZEN⁸ the function of the *spleen* appears to stand in a certain relationship to the decomposition of the zymogen, for the pancreas of a starving dog was inactive until treated with the spleen-substance of a dog which had been fed.

¹ Albertoni, *Maly's Jb.*, viii., 127.

² Kühne, *Verh. naturh. med. Ver. Heidelberg*, New Series, iii.

³ Halliburton and Brodie, *Journ of Phys.*, xx., 97.

⁴ Edkins, *Journ. of Physiol.*, xii., 193.

⁵ Roberts, *Proceed. Royal Society*, xxxii., 145.

⁶ Heidenhain, *Pflüg. A.*, x., 581.

⁷ Podolinsky, *Beiträge zur Kenntniss d. pancreat. Eiweissferm.* Diss. Breslau, 1876.

⁸ Herzen, *Centralbl. f. med. Wiss.*, 1877, 435.

The pancreas acts more energetically in conjunction with the spleen-substance than by itself; this action is not to be attributed to a sort of stimulation of the trypsinogen by the hæmoglobin-oxygen, since arterial blood is inactive, whilst the venous blood of the spleen saturated with carbon dioxide is active (HERZEN¹).

According to ALBERTONI² this zymogen is present in the foetus during the last months.

Products of Trypsin Digestion.—Trypsin acts very energetically on all albuminous substances, including their simplest representatives, the *protamines* (KOSSEL and MATTHEWS³). The only successful attempt to decompose simpler substances by trypsin is that of BLANK,⁴ who claims to have decomposed hippuric acid; on the other hand, GULEWITSCH⁵ did not succeed in accomplishing this beyond doubt in the case of any single simpler compound.

According to HERMANN,⁶ a *globulin* coagulating at 55° to 60° C. is produced in the digestion of unboiled fibrin with trypsin, whilst the *paraglobulin* found with it (OTTO,⁷ HASEBROEK⁸) represents an impurity of the fibrin. There are then formed as by-products albumoses and peptones; in a vigorous action, however, two main groups of bodies are formed—viz., *amido-acids* and the more recently recognised *hexone bases*—*lysine*, *arginine*, and *histidine*. *Ammonia* is also produced (HIRSCHLER,⁹ STADELMANN,¹⁰ KUTSCHER,¹¹) together with *tryptophan*.

Before dealing more closely with the characteristics of these decomposition-products, we must take into consideration the dispute which has recently arisen as to the existence of the so-called anti-peptone of Kühne.

As we have related in the chapter on *pepsin*, KUHNE believed that it was necessary to assume the existence of two kinds of peptones; he concluded that the hemi-peptone was decomposed further by the trypsin, whilst the *anti-peptone* resisted the action of this enzyme. He made no definite assertions about its more special properties, and, indeed, hardly regarded it as a chemical

¹ Herzen, *Annali di Chim. e Farm.*, 1888, 302.

² Albertoni, *Maly's Jb.*, viii., 254.

³ Kossel and Matthews, *Z. physiol. Ch.*, xxv., 190.

⁴ Blank, quoted by Nencki, *Arch. f. exp. Path.*, xx., 377.

⁵ Gulewitsch, *Z. physiol. Ch.*, xxvii., 540.

⁶ Hermann, *Z. physiol. Ch.*, xi., 508.

⁷ Otto, *Z. physiol. Ch.*, viii., 129.

⁸ Hasebroek, *ibid.*, xi., 348.

⁹ Hirschler, *Z. physiol. Ch.*, x., 302.

¹⁰ Stadelmann, *Z. f. Biol.*, xxiv., 226.

¹¹ Kutscher, *Endprod. d. Trypsinverd. Habilit. - Schr.*, Strassb., 1889, 10.

individual. On the other hand, SIEGFRIED¹ and BALKE² maintained the chemical individuality of "anti-peptone," which, in their opinion, was identical with Siegfried's carnic acid.

Whilst KÜHNE³ himself makes varying statements as to the formula and reactions of anti-peptone, Siegfried and Balke assign to it the formula $C_{10}H_{15}N_3O_5$.

They did not succeed in eliminating a small amount of sulphur, which they regarded as an impurity. FRAENKEL⁴ describes a method of preparing anti-peptone free from sulphur.

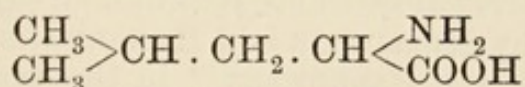
KUTSCHER⁵ has now succeeded in proving that considerable quantities of hexone bases are split off from the so-called *anti-peptone* by precipitation with phosphotungstic acid, whilst leucine, tyrosine, and other amido-acids are obtained from the non-precipitable residue. He has also successfully defended this important result against the attacks of Siegfried.

He has, moreover, succeeded in eliminating, to all but a very trifling extent, in the digestion of pancreas substance with trypsin, the *biuret reaction*, which has been described as specially characteristic of anti-peptone. From all this, it appears probable that Kühne's anti-peptone must disappear from our conception of trypsin digestion, and that the decomposition of albuminous substances by trypsin takes place in a perfectly analogous manner to that caused by strong sulphuric acid.

In detail, the decomposition-products of the albuminous substances are as follows:—

Amido-Acids.—Leucine⁶ was first discovered in the pancreas by VIRCHOW.⁷ Subsequently it was found in many organs, and also in invertebrates and plants.

It is a normal product in every energetic decomposition of albuminous bodies, horn substance, elastin, &c. *Leucine* is an amido-caproic acid, probably the α -acid of the formula⁸



¹ Siegfried, *Du Bois Arch. f. Phys.*, 1894, 401. *Z. f. physiol. Ch.*, xxi., 360.

² Balke, *Z. physiol. Ch.*, xxii., 248.

³ Kühne, *Z. f. Biol.*, xxii., 450; xxviii., 571; xxix., 1, 308.

⁴ Fraenkel, *Wien. med. Blätter*, 1896, 708.

⁵ Kutscher, *Z. physiol. Ch.*, xxv., 195; xxvi., 110. Also, *Die Endprod. d. Trypsinverdauung Habil.-Schr.*, Strassburg, 1899.

⁶ For a full description and bibliography of *leucine*, see Gamgee, *Phys. Chem. d. Verdauung*, 244.

⁷ Virchow, in his *Archiv.*, vii., 580.

⁸ Hüfner, *J. pr. Ch.* (New Series), i., 6. Schulze and Likiernik, *Ber. d. d. chem. Ges.*, xxiv., 669. Gmelin, *Beitr. z. Kenntn. d. Leucins*, Diss. Tübingen, 1892 (very complete bibliography).

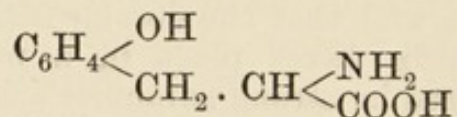
The vegetable and animal leucines are probably stereo-isomeric, possessing the same constitution.

It has been prepared synthetically from iso-valeric aldehyde-ammonia and hydrocyanic acid (HÜFNER, *loc. cit.*). The synthetical product is optically inactive; the natural one, dextro-rotatory. *Penicillium glaucum* removes the dextro-rotatory form from a racemic mixture, and leaves behind only a lævo-rotatory variety.

Isomeric leucines have been described by R. COHN,¹ and HÜFNER and NENCKI.² The ordinary leucine crystallises in characteristic globular masses, melts at about 170° C., and sublimes unchanged in fine woolly masses. It is fairly soluble in water, but dissolves with difficulty in alcohol. Its *phenyl hydantoin* and the ethyl ester of its hydrochloride are characteristic (RÖHMANN³).

Tyrosine.⁴—In contradistinction to leucine, tyrosine is never⁵ met with in the living tissue of higher animals, except in certain diseases, but, on the other hand, it is found in many invertebrate animals. It is produced in the decomposition of all proteids, with the exception of *gelatin*.

It is a *para-oxyphenyl-amido-propionic acid* (synonyms, oxy-phenylalanine, α -amido-para-hydrocumaric acid).



It has been prepared synthetically by ERLÉNMEYER and LIPP,⁶ but in an optically inactive form, which was first decomposed by E. FISCHER⁷ into its optical components. It is soluble with great difficulty in water, and is insoluble in alcohol. It crystallises in fasciculated needles, that is, grouped in bundles. On boiling with Millon's reagent (mercuric nitrate solution) it gives the red coloration characteristic of proteids.

Aspartic Acid, as a decomposition product of proteids was discovered by RITTHAUSEN and KREUSSLER⁸ (in vegetable proteids), and also by HLASIWETZ and HABERMANN.⁹ It was found by RADZIEJEWSKI and SALKOWSKI¹⁰ in the tryptic digestion of

¹ Cohn, *Z. physiol. Ch.*, xx., 203.

² Nencki, *J. pr. Ch.* (New Series), xv., 390.

³ Röhmnn, *Ber. d. d. chem. Ges.*, xxx., 1978; xxxi., 2188.

⁴ For a full account of *tyrosine* also, reference must be made to Gamgee, *loc. cit.*, 256.

⁵ Radziejewski, *Virch. A.*, xxxvi., 1. Kühne, *Untersuch. physiol. Institut. Heidelb.*, i., 317.

⁶ Erlenmeyer and Lipp, *Ber. d. d. chem. Ges.*, xv., 1544.

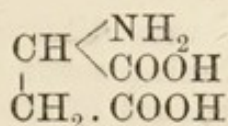
⁷ E. Fischer, *Ber. d. d. chem. Ges.*, xxxii., 2451, 1899.

⁸ Ritthausen and Kreussler, *J. pr. Ch.* (New Series), iii., 314.

⁹ Hlasiwetz and Habermann, *Lieb. Ann.*, clxx., 304; clxxix., 150.

¹⁰ Radziejewski and Salkowski, *Ber. d. d. chem. Ges.*, vii., 1050, 1874.

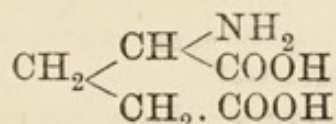
fibrin, and in that of gluten by v. KNIERIEM.¹ It is an amido-succinic acid.



It dissolves readily in hot water, but with difficulty in cold, and is insoluble in alcohol. It is laevorotatory. Its copper salt is characteristic. The inactive synthetic aspartic acid, as also E. FISCHER'S² glutamic acid, has been decomposed into the two optical components, by means of the brucine salt of its benzoyl compound.

Glutamic Acid was isolated from vegetable proteins by RITTHAUSEN and KREUSSLER,³ and from animal proteins by HLASIWETZ and HABERMANN⁴ by means of hydrochloric acid and stannous chloride, and by KUTSCHER⁵ with the aid of sulphuric acid. In pancreatic digestion it was separated by v. KNIERIEM.¹

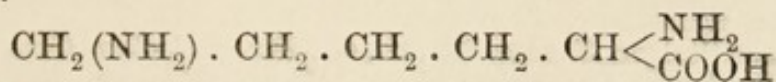
It is an amido-glutaric acid.



It dissolves with difficulty in water, and is insoluble in alcohol. M. pt. 135° to 149° C. It, too, forms a characteristic copper salt.

The Hexone Bases.—The portion of the tryptic decomposition products capable of being precipitated by phospho-tungstic acid contains three bases which Kossel has grouped together as *hexone bases*—viz., lysine, arginine, and histidine.

Lysine was discovered by DRECHSEL⁶ as a decomposition product of casein, and was subsequently examined more fully by him and his pupils. It was found by HEDIN⁷ in the products of tryptic digestion. It is isolated most readily in the form of its *picrate* (KOSSEL⁸). It is a 1·5 diamido-caproic acid (ELLINGER⁹), since on putrefying it yields *cadaverine* (pentamethylene-diamine).



It is dextro-rotatory.

¹ v. Knieriem, *Zeitsch. f. Biol.*, xi., 198.

² E. Fischer, *Ber. d. d. chem. Ges.*, xxxii., 2451, 1899.

³ Ritthausen and Kreussler, *J. pr. Ch.* (New Series), iii., 314.

⁴ Hlasiwetz and Habermann, *Lieb. Ann.*, clxx., 304; clxxix., 150.

⁵ Kutscher, *Z. physiol. Ch.*, xxviii., 123.

⁶ Drechsel, *Abbau der Eiweisskörper* (Comprehensive research). *Du Bois Arch.*, 1891, 248; cf. *Ber. d. d. chem. Ges.*, xxv., 2454.

⁷ Hedin, *Du Bois Arch.*, 1891, 273.

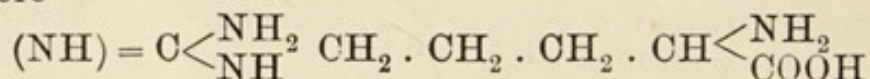
⁸ Kossel, *Z. physiol. Ch.*, xxvi., 586.

⁹ Ellinger, *Ber. d. d. chem. Ges.*, xxxii., 1899.

It forms a di-benzoyl compound (*lysuric acid*), which has a characteristic acid barium salt (DRECHSEL¹).

Arginine, $C_6H_{14}N_4O_2$, was discovered by SCHULZE and STEIGER² in germinating lupine seeds (1886). HEDIN found it to be a decomposition product of albuminous bodies³ and of horn substance⁴ and (KOSSEL⁵) of protamines. KUTSCHER⁶ obtained it in artificial digestions with trypsin. With regard to its constitution we can form a conclusion from the researches of SCHULZE and LIEKERNIK,⁷ SCHULZE and WINTERSTEIN,⁸ and ELLINGER⁹ (indirectly through his elucidation of the nature of *ornithine*).

According to the latter, arginine is to be regarded as the kreatine of 1.4 diamido-valeric acid (*ornithine*). Its formula is therefore



On decomposition with baryta water it yields *urea* and diamido-valeric acid (*ornithine*).

SCHULZE and WINTERSTEIN¹⁰ have prepared it synthetically from *ornithine* and *cyanamide* by a method analogous to the synthesis of kreatine. The vegetable arginine differs somewhat from the animal compound.

Arginine and its salts were thoroughly examined by GULEWITSCH.¹¹ The base itself crystallises in rosette-like groups, which melt at 207° C. The double acid salt formed with silver nitrate is specially characteristic.

Histidine.—Histidine was obtained by KOSSEL¹² in the decomposition of *styrine* by dilute sulphuric acid, and was subsequently shown by SCHULZE and WINTERSTEIN¹³ to be a decomposition product of albuminous bodies. In pancreatic digestion it was discovered by KUTSCHER.¹⁴ With regard to its chemical properties, there is still but little known.¹⁵ It appears to have the

¹ See Clara Willdenow, *Z. physiol. Ch.*, xxv., 523.

² Schulze and Steiger, *Z. phys. Ch.*, xi., 43; *Ber. d. d. chem. Ges.*, xix., 1177.

³ Hedin, *Z. physiol. Ch.*, xxi., 155. ⁴ Hedin, *ibid.*, xx., 186.

⁵ Kossel, *ibid.*, xxii., 184; Kossel and Matthews, *ibid.*, xxv., 190.

⁶ Kutscher, *ibid.*, xxv., 195.

⁷ Schulze and Likiernik, *Ber. d. d. chem. Ges.*, xxiv., 2701.

⁸ Schulze and Winterstein, *Z. physiol. Chem.*, xxiii., 1; *Ber. d. d. chem. Ges.*, xxx., 2879.

⁹ Ellinger, *Ber. d. d. chem. Ges.*, xxxi., 3183.

¹⁰ Schulze and Winterstein, *Ber. d. d. chem. Ges.*, xxxii., 3191, 1899.

¹¹ Gulewitsch, *Z. physiol. Chem.*, xxvii., 178, 368.

¹² Kossel, *Z. physiol. Ch.*, xxii., 76. ¹³ Hedin, *ibid.*, xxii., 191.

¹⁴ Schulze and Winterstein, *Z. physiol. Ch.*, xxviii., 459, 1899.

¹⁵ Kutscher, *ibid.*, xxv., 195.

¹⁶ See Kossel and Kutscher, *ibid.*, xxviii., 382, 1899.

formula $C_6H_9N_3O_2$. It is separated in the form of its silver compound.

Glycocoll.—*Amido-acetic acid*, $CH_2 < \begin{smallmatrix} NH \\ COOH \end{smallmatrix}$

has long been known as a decomposition product of *gelatin*, but has only recently been discovered by SPIRO,¹ with the aid of a new method, among the decomposition products of true albuminous bodies.

Tryptophan.—Under the name *tryptophan*,² formerly *protein-chromogen*, are grouped certain substances, the nature of which was long unknown, which are produced in the decomposition of albuminous bodies, including that effected by trypsin, and are characterised by the fact that they give a red coloration with halogens (chlorine, bromine). This red coloration was first observed by TIEDEMANN and GMELIN.³ CLAUDE BERNARD⁴ next showed that this reaction first appeared after the commencement of decomposition (putrefaction), but confused it with the similar reaction given by *indol* (or *naphthylamine* as indol was at first regarded, cf. HEMALA),⁵ which is only formed in putrefaction—an error which was afterwards pointed out by KÜHNE.⁶

NENCKI⁷ investigated the bromine compounds and found that at least two substances are formed, which probably belong to the indigo group. On fusion with potassium hydroxide he obtained pyrrol, indol, &c. KURAJEFF⁸ on examining the bromine product obtained three distinct substances. The chlorine compound was examined by BEITLER,⁹ who succeeded in preparing from it, by means of silver oxide, a basic substance free from chlorine. The so-called tryptophan dissolves with difficulty in water, alcohol, and ether, but more readily in amyl alcohol.⁵

Differences between the Tryptic Digestion of Albuminous Substances and of Albuminoids.—In general the tryptic decomposition process follows practically the same course. Only the quantitative proportions of the different constituents vary. *Gelatin* is very readily attacked by trypsin, and its use is therefore recommended by FERMI¹⁰ for the detection of trypsin.

¹ Spiro, *Z. physiol. Ch.*, xxviii., 174, 1899.

² The name is due to Neumeister, *Z. f. Biol.*, xxvi., 329.

³ Tiedemann and Gmelin, *Verdauung nach Versuchen*, Heidelberg, 1826, 31.

⁴ Cl. Bernard, *Comptes Rend.*, 1856, i., Suppl., 403.

⁵ Hemala, in Krukenberg's *Chem. Unters. z. wissenschaft. Medizin*, ii., 119, 1888.

⁶ Kühne, *Ber. d. d. chem. Ges.*, viii., 206.

⁷ Nencki, *Ber. d. d. chem. Ges.*, xxviii., 560.

⁸ Kurajeff, *Z. physiol. Ch.*, xxvi., 501.

⁹ Beitler, *Ber. d. d. chem. Ges.*, xxxi., 1604; Stadelmann, *Z. f. Biol.*, xxvi., 491.

¹⁰ Fermi, *Maly's Jb.*, 1892, 592.

The tryptic digestion of *casein* was first investigated by SEBELIEN. BIFFI¹ then found that casein was entirely digested with the exception of a trifling residue nearly free from phosphorus. He also found fairly large quantities of *tyrosine*, *casein-albumose*, and *casein-antipeptone*. The phosphorus was partially split off as phosphoric acid and partially converted into another firmly combined form. As regards the tryptic digestion of other *nucleo-proteids* and *nucleïns* no accurate results have been published, but KUTSCHER² found in the auto-digestion of the pancreas *xanthine*, *hypoxanthine*, and *guanine*, which would be derived from the protoplasmic substances of the cell. On the other hand BOKAY³ asserts that nucleïns resist tryptic digestion.

BABCOCK and RUSSELL⁴ claim to have discovered in ripening cheese a proteolytic ferment with a specific action on *paracasein*, to which they have given the extremely unsuitable name of *galactase*. It is stated to work best in weakly alkaline media, and to resemble trypsin, though not to be identical with it.

The Auto-Digestion of Organs.—Under the head of the proteolytic ferments falls most suitably a description of a remarkable phenomenon, in which similar reactions occur—i.e., the so-called *auto-digestion of organs*—which has been investigated notably by SALKOWSKI and his pupils. In such cases there is a slow decomposition of albuminous substances, although putrefaction is excluded (by means of chloroform water), which SALKOWSKI first studied in the case of *yeast*,⁵ but subsequently also in the organs of animals.⁶ He digested the finely-divided organs containing fresh blood with ten times their quantity of chloroform water, and found that in these self-digesting organs *leucine* and *tyrosine*, *reducing sugars*, &c., were present, all of which were absent in the fresh organs, and, moreover, could not be detected when the organs were *boiled before* the digestion, thus causing the suppositious enzymes to disappear.

He also found more free *nucleïn bases* than in fresh extracts, by which result he was able to confirm the statements of SALOMON.⁷

The assumption of proteolytic ferments was then more firmly established by SCHWIENING,⁸ who obtained the same results with extracts *free from cells*.

¹ Biffi, *Virch. A.*, clii., 130.

² Kutscher, *Endprod. d. Trypsinverd.*, 1899. Habilit-Schr.

³ Bokay, *Z. physiol. Ch.*, i., 157.

⁴ Babcock and Russell, *Chem. Centralbl.*, 1900, i., 430.

⁵ Salkowski, *Z. physiol. Ch.*, xiii. ⁶ *Id.*, *Z. f. klin. Med.*, xvii., Suppl.

⁷ Salomon, *Du Bois Arch. f. Phys.*, 1881, 361.

⁸ Schwiening, *Virch. A.*, cxxxvi., 1894.

BIONDI,¹ too, found in "digested" calves' livers *xanthic bases*, but not in control experiments, and considered that he was justified in assuming that they were possibly derived by decomposition from the *nucleo-proteids*. He also found *albumoses* and *leucine* in the main experiment. This process differed from tryptic digestion with pancreas powder by the fact that in the latter *peptones* and the *tryptophan reaction* could be observed, whilst they were absent in the *auto-digestion*, and that the tryptic digestion dissolved more organic matter, but specially by the pancreas ferment not possessing the capacity for decomposing nucleïns.²

Sodium fluoride and thymol proved to be less effective than chloroform. Alkalies retard the auto-digestion (SCHWIENING); the effect of acids is difficult to determine, but they appear not to interfere with it, in slight degrees of concentration at least.

JACOBY³ found glycocoll in the auto-digestion of the liver. He was able to "salt out" the ferment by means of ammonium sulphate. In cases of phosphorus poisoning the auto-digestion is said to be greatly accelerated, and to take place even during life.

¹ Biondi, *Virch. A.*, cxliv., 373, 1896.

² We have, however, seen above that trypsin also decomposes nucleïns, at any rate in the auto-digestion of the pancreas.

³ *Z. physiol. Ch.*, xxx.

CHAPTER XI.

THE BACTERIOLYTIC AND HÆMOLYTIC FERMENTS.

PROTEOLYTIC ferments undoubtedly play a decisive part in the phenomena of *bacteriolysis* and *hæmolysis*, which, of late years, have received special attention. These processes, however, are of such a distinct nature that we must speak of them separately.

For a long time past, the researches of HANS BUCHNER¹ and his pupils have made us acquainted with the power of blood serum to destroy intruding bacteria. To explain this phenomenon, the existence of special protective substances, *alexines*, was assumed, and to these great importance was assigned in the production, both of inherited and acquired *immunity*. It is out of my province to discuss the whole question of the alexines and the voluminous literature which deals with them, the more so since Buchner himself has quite recently expressed the opinion that, in these processes, we have to deal with the actions of *proteolytic ferments*. We have thus only the task of describing the characteristic nature and mode of action of these *ferments*.

The ferments which effect the destruction of bacteria which have forced their way into the circulation of the blood are not directly analogous to the ordinary proteolytic ferments which act upon all albuminous substances, but are sharply differentiated from them by their *marked specific* character.

Although the normal blood-serum also contains a very trifling amount of simple proteolytic ferments and this fact can be used with advantage as an illustration, as we shall see subsequently, yet the presence of these enzymes is not enough to enable us to offer an explanation of these specific bacteriolytic ferments.

We have here to deal with the fact that, in the active immunisation of an animal, particularly against *cholera* and *typhus*, bacteriolytic substances are formed in the blood-serum, which are capable of destroying, *within the body only*, those bacteria against the action of which the animal was rendered

¹ Buchner, *Münch. med. Woch.*, 1899, 39-40.

immune; thus the *cholera antiserum* only destroys the *cholera vibrios*, that of *typhus* only the *typhus bacilli*.

We might thus assume that the immune-serum of cholera contains a substance which, as a specifically directed ferment, possesses the power of destroying the cells of the cholera vibrios. This would imply the presence of proteolytic ferments endowed with so supersubtle a power of differentiation as compared with other ferments, that whereas the latter are specific only for large groups of substances, the *cholera immune ferment* is so sensitive to the refined differences between cell protoplasms that it will not attack the typhus bacilli.

A very remarkable and theoretically important addition to our knowledge was made by the noteworthy experiments of PFEIFFER.¹ Pfeiffer's phenomenon teaches us the following facts:—

When the serum of an animal rendered immune against cholera is tested with reference to its bacteriolytic power *outside the body*, it is found that this power is really *no greater* than the trifling bacteriolytic capacity of *normal serum*, which, as was mentioned above, is to be attributed to the presence of proteolytic ferments in the serum. Even of this slight power the serum can be completely deprived, *e.g.*, by heating to 55° C., so that it is then powerless against the vibrios.

But, if this inactive serum be re-introduced into the organism—*e.g.*, if it be injected together with living vibrios into the abdominal cavity—it immediately develops its intense action upon the vibrios and *only* on these, typhus bacilli, for example, remaining unaltered. Thus, the latent capacity of the immune-serum is renovated within the organism by some agency. The experiments of METSCHNIKOFF² and BORDET³ prove that we have not to deal here with some possible *vital* capacity of the organism. For the same result can be obtained without having recourse to the living organism by adding *fresh normal* blood-serum to the inactive immune-serum. This addition is sufficient to impart the power of bacteriolysis in its full strength to the previously inactive serum. It is thus evident that the *latent* proteolytic power of immune-sera is rendered active again by the addition of an agent normally present in blood-serum.

One might incline to the view that a *proferment* is possibly present in the inactive immune-serum, and is rendered active by a zymoplastic agent of the blood-serum. Yet there is no

¹ Pfeiffer, *D. med. Woch.*, 1896 to 1898.

² Metschnikoff, *Ann. Inst. Pasteur*, ix., 1895.

³ Bordet, *Ann. Inst. Pasteur*, ix., 1895. Quoted by Ehrlich, *loc. cit.*

ground for such a supposition. We know that *dilute acids* are pre-eminent as zymoplastic agents, whilst the blood-serum is alkaline; and, moreover, we never find a zymoplastic activity in the blood-serum, whilst, on the contrary, we very frequently observe that it has an action *directly prejudicial to ferments*.

Before turning to the attempts which have been made to explain this phenomenon we must first describe the very similar and theoretically important phenomenon of *hæmolysis*. BORDET¹ first discovered that on injecting the blood of a rabbit into the circulatory system of a guinea-pig the serum of the latter animal acquires the capacity of *dissolving in vitro* the red corpuscles of the rabbit. This capacity can be destroyed by heating the serum to 55° C., but is again restored by the addition of normal serum. The case is thus perfectly analogous to that of the *bacteriolysines*. EHRLICH and MORGENROTH² have repeated and extended these experiments. They injected sheep's blood into a goat, with the result that the goat's serum acquired the capacity of dissolving the erythrocytes of the sheep. This power was lost on heating, but *restored* by the addition of normal serum; this latter serum, however, must be fresh, since it otherwise loses the power of reacting, even when it has been kept in the dark and on ice. From these experiments Ehrlich comes to the following conclusions:—

Both in bacteriolysis and in hæmolysis we have an immunisation against an intruding *substance injurious to the protoplasm*, whether it be a bacterium or an erythrocyte. The conveyor of this immunising action is an *immune-substance*. This immune substance is perfectly analogous to the anti-toxines, and Ehrlich explains its development, like that of the anti-bodies, by the *side-chain theory*. This, as the reader will be aware, postulates that the injurious substances which force their way into the organism, whether toxines or organised cells, attach themselves with the aid of their *haptophore* groups to the side chains of the attacked cells, and that the side chains produced in excess then circulate through the blood and form the specific *immune-substances*.³ These are partially of an *anti-toxic* nature, *i.e.*, capable of paralysing the *poisons* which have forced their way into the system or have been excreted by the bacteria there. The immune-sera, however, which have bacteriolytic powers, do

¹ Bordet, *Ann. Inst. Pasteur*, xii.

² Ehrlich and Morgenroth, *Berl. klin. W.*, 1899, 2, 22.

³ As regards the side-chain theory, I must refer the reader to Ehrlich's publications, especially in the *Klin. Jahrb.*, vi. See also my abstract in the *Biol. Centralbl.*, 1899, 799.

not possess such properties. PFEIFFER¹ has shown that they are powerless against the action of the poison—*e.g.*, of cholera vibrios. If the animal used in the experiment be inoculated with virulent cholera cultures followed after some hours by immune-serum, the animal dies from the *effects of the poison*, although the phenomena of *bacteriolysis* are present to their full extent.

In its ordinary condition, however, but specially after heating to 55° C., the serum containing the immune body exhibits no *bacteriolytic* properties; these, rather, first appear when to the immune substance in the fresh serum, inactive in itself, is added a certain something to which Ehrlich has given the name *addiment*, which involves no assumption. This addiment, then, we must regard as a *proteolytic enzyme*.

In the conception of his side-chain theory Ehrlich imagines the following process to occur:—

The *immune-substance* first attaches itself by means of the haptophore groups to the substance which injures the protoplasm. By itself it has no destructive influence on the cell, but, on the other hand, it possesses the power of attaching to itself, by means of another haptophore group, the *proteolytic enzyme*, or addiment, and thus bringing the active or *zymophore*² group of the latter into contact with the injurious substance, the destruction of which it in this way brings about. The *specific function* of the immune body lies in the fact that it concentrates upon the intruder, to which it is specifically adapted, the enzyme which is already present in the blood, though scantily and in a dilute condition; hence the *proteolytic ferment* need not be specific. In support of this conception Ehrlich was able to show that the addiment-free immune-substance (after being heated to 55° C.) combined *quantitatively* with the erythrocytes *adapted to it*, whilst the latter absorbed no trace of "addiment" from pure solutions of that substance. But when the erythrocytes are introduced into a solution which contains the immune body *and* the addiment a part of the latter is also taken into combination—*i.e.*, since it does not directly combine with the blood-corpuscles it must be indirectly attached to them—*i.e.*, *through the agency of the immune body*.

We have thus to assume for the explanation of bacteriolysis

¹ Pfeiffer, *D. med. Woch.*, 1896, Nos. 7 and 8.

² This terminology is not used by Ehrlich. I have *only* employed it as a mental picture of the analogy with the toxins. Moreover, I observe that Ehrlich's pupil, Morgenroth, also makes use of it (*cf. Bact.*, 1899, *loc. cit.*), though in discussing rennet.

and hæmolysis that a *non-specific* proteolytic ferment is so concentrated through the agency of a *specific* intermediate substance, the *immune body*, that it can now develop its fermentative, albumin-dissolving action. This conception has, however, been opposed on different sides. Emmerich and Löw¹ have found that the exclusion of the bacteriolysis in Pfeiffer's experiment does not succeed when the immune-serum is brought into contact with the vibrios in the absence of oxygen. In this case, then, a *fresh* addition of addiment—i.e., of proteolytic enzyme—is not necessary. It is, however, a simple matter to explain this by the assumption that the enzyme *already present* is not so rapidly destroyed in the absence of oxygen as when it is present. They do not state whether bacteriolysis still occurs after the heating to 55° C., in which case the *original* addiment would be destroyed.

BUCHNER² opposes Ehrlich's conclusions from another point of view.

He contends for a complete separation of the *immune body* from the *proteolytic ferment*, the *alexine*. While he fully confirms Ehrlich's results, he draws other conclusions from them. The immune body, which resists heating to 55° C., is *specific*, and combines with the substratum; the ferment destroyed by the heat—the *alexine* = Ehrlich's *addiment*—is *not* specific, and so does not combine with the substratum. As regards the non-specific character of the ferment, which, indeed, Ehrlich also assumes, he gives in addition the striking proof that even the serum of a *third* and different species of animal can act as a proteolytic ferment, and that the *serum belonging to* the erythrocytes which are to be attacked can also fulfil this function. All this substantially agrees with Ehrlich's views. Ehrlich, too, asserts, *without exception*, the specific nature of the immune body: the difference lies in the facts that Ehrlich, on the one hand, ascribes to the *immune body as such* no injurious influence upon the intruding substance, and that he *only* regards the activity of the ferment as being induced *through the agency of the anti-body*, whereas Buchner is willing, it is true, to make a sharp distinction between the two processes of the *fixation* by the specific immune body, and the *solvent action* of the alexine, but instead ascribes to the immune body, as such, an influence on the protoplasm, as well as a *determining* influence upon the activity of the alexine.

The bacillus or erythrocyte is first so influenced by the action

¹ Emmerich and Löw, *Z. f. Hyg.*, 1899, 1.

² Buchner, *Münch. med. Woch.*, 1900, 277.

of the anti-body that it falls a prey to the alexine.¹ Now, this would really only be a dispute as to terms, in which Ehrlich gives a fuller definition of the "determining momentum" in stating that the ferment is directed towards the cell through the agency of the immune body *in an absolutely definite manner*. The difference lies deeper, however. Buchner sees in the phenomenon of *agglutination* an actual directly injurious influence of the *immune body by itself* upon the cell. In fact, the sera when freed from the addiment still show the property of agglutinating the corpuscles of the blood (BORDET, *loc. cit.*), and the immune-sera behave in a similar manner towards *bacteria*. In this alteration of the upper layers of the cell-protoplasm Buchner detects the manifestation of an *injurious force of the pure immune body*. The cells thus injured are represented as being then first accessible to the attack of the alexines, whilst the latter *cannot* attack cells which have not been so prepared. To this Ehrlich objects that it is not permissible to identify *agglutinines* with the specific anti-bodies without further proof, since the two processes, *bacteriolysis* and *agglutination*, are not always inseparably bound up with one another.

Moreover, assuming that the agglutination is really a *constant function* of the immune body, it is not comprehensible why the fixation of the latter to the cell protoplasm, which doubtless occurs, and has also been assumed by Buchner, should not be accompanied by *changes* in the cell-structure capable of *being recognised under the microscope*.

On the other hand, against Buchner's assumption that the action of the alexine is a phenomenon which, although attended by a preliminary action of the immune body, is yet independent of the latter, may be urged the fact established by Ehrlich of the indirect combination of the ferment with the substratum through the medium of the immune body.

If, then, Ehrlich's theory be compared from this point of view with Buchner's views, it is evident that there is essentially *no very profound* difference between them; if we exclude the, as yet unsettled, question of the definite existence of *agglutinines*, we find in both *specific anti-bodies* and *non-specific proteolytic enzymes*. It is only by the *combined* action of *both* agents that the *plasmato-lysis*² is brought about. The only important difference then

¹ Cf. Trumpff, *Z. f. Hyg.*, xxxiii., 70.

² It would be distinctly advisable to find a common name for the very similar phenomena of bacteriolysis and hæmolysis and some other allied processes. *Plasmolysis* and *Cytolysis* have unfortunately been applied by the botanists to other phenomena; possibly the word *plasmato-lysis* might be used.

remaining is that Buchner assumes only a certain weakening of the cell structure, which prepares the way for the action of the ferment, whilst Ehrlich, reasoning from his side-chain theory, the ultimate consequences of which Buchner does not admit, constructs a pictorial representation, which is, of course, purely hypothetical, of the reciprocal relations of the immune body and the ferment.

On one point Buchner is obviously completely right—i.e., in offering an uncompromising resistance to the expression, *restoration of the activity of the immune body*. The specific bacteriolytic or hæmolytic *function* is, as the results on both sides have shown, not the product of a single specific bactericidal *substance*. The action, in any case, is brought about by the combined activity of a specific immune body, capable neither of *being rendered inactive* nor of having its *activity restored*, and of a non-specific proteolytic ferment which is equally incapable of *having its activity restored*, but which can be readily destroyed and can be replaced by fresh material.

But such a *restoration of activity* is not even assumed by Ehrlich, but only a *production of activity* through the agency of a non-specific proteolytic ferment of the serum, combined, in some way or other with the immune body.

To my mind, no deep-seated difference between the views of Ehrlich and of Buchner appears to be involved in this point.

The proteolytic ferment, Buchner's *alexine* and Ehrlich's *addiment*, is thus present in every fresh serum. With regard to its nature, we can say no more than that it is far more sensitive to the action of heat, as also of light and air, and even to the alkalinity of the blood, than any other known ferment. No attempts have as yet been made to isolate it.

The question of the significance of *leucocytes* in the formation of bacteriolytic substance has given rise to particularly vigorous discussion. PFEIFFER¹ was able to show that bacteriolytic processes did not occur with greater intensity in liquids rich in leucocytes than in ordinary serum, and MOXTER,² who studied the process of dissolution under the microscope, was able to confirm this. On the other hand, BUCHNER,³ for instance, has shown that it is extremely probable that the *ferments* required for the bacteriolysis (*alexines*) have their origin, for the most part at least, in the leucocytes, for both *pus* and other media, rich in leucocytes, also exhibited vigorous proteolytic properties

¹ Pfeiffer, *D. med. Woch.*, 1896, Nos. 7 and 8.

² Moxter, *D. med. Woch.*, 1899, No. 42 (Bibliography).

³ Buchner, *Münch. med. Woch.*, 1900, 277.

(LEBER¹). Possibly we might be right in assuming that, although the liquids rich in leucocytes contain *more* alexines, a given quantity of the immune body also finds in the *serum* sufficient ferment to effect a *maximum bacteriolysis*, and that an excess of ferment does not further intensify the process. In that case, our special interest in this question would be at an end; for the other special factors of this process, the *immune bodies*, are not *ferments*. They are, however, so closely bound up with the whole question of plasmolysis that we will briefly add some further remarks about them.

We will not open up any further here the question of their origin. It is entirely included in the wider dispute as to the origin of the anti-bodies. Whilst Ehrlich and others assume that the anti-bodies are products formed for the protection of the attacked *organism*, Buchner and his supporters regard them as modified bacterial substances. As regards their *place of origin*, PFEIFFER and MARX² have proved that the *spleen* and *marrow of the bones* are of primary importance in this respect.

We must not confuse with these specific bacteriolytic substances the simple *bactericidal* substances originating from the *leucocytes*, the significance of which has been clearly established in the case of *nucleic acid*, for example, by A. and H. KOSSEL.³

¹ Leber, *Entstehung der Entzündg.*, Leipzig, 1891. Quoted by Buchner, *loc. cit.*

² Pfeiffer and Marx, *Z. f. Hyg.*, xxvii.

³ A. and H. Kossel, *Z. f. Hyg.*, xxvii. (Bibliography).

CHAPTER XII.

VEGETABLE PROTEOLYTIC ENZYMES.

THE discovery in the germinating parts of plants of soluble nitrogenous substances, which were then regarded as the degraded products of the decomposition of albumin by chemical or fermentative actions, was made long ago. The first were *asparagin*, which VAUQUELIN and ROBIQUET¹ found in asparagus, and *glutamic acid*, first discovered by SCHULZE and BARBIERI in pumpkin seeds, where it is accompanied by asparagine and ammonia. Subsequently this field of research was explored, notably by E. SCHULZE² and his pupils.

When, then, GORUP-BESANEZ³ discovered *leucine*, which is one of the main decomposition-products of albuminous substances, accompanying asparagine and glutamic acid, and *peptones* were found by SCHULZE in germinating lupine seeds, it was not a great step to infer that in the *embryos of plants*, which, like the animal organism, must maintain themselves from stored-up nutriment without assimilation, *proteolytic* ferments were present in addition to the long-known diastatic ferments. In fact, GORUP-BESANEZ⁴ shortly afterwards succeeded in isolating diastatic and proteolytic ferments, which were simultaneously present, from germinated vetches, and also from hemp, linseed, and barley.⁵ In the last-named he found no leucine and tyrosine, but only peptones. From *germinating lupine seeds*, however, he was unable to isolate any ferment. On the other hand, GREEN⁶ obtained from the sprouting seeds of *Lupinus hirsutus*, an enzyme which produced the "so-called" peptone, *leucine* and *tyrosine*, but was only active in acid solution. He assumed that a zymogen was present in the *quiescent* seeds.

¹ Quoted by Piria, *Annal. d. Chim. et Phys.* (iii.), xxii., 160.

² Vide Schulze and his pupils, *Landwirthsch. Jahrb.*, v., 281; vi., 681; vii., 411; ix., 689 (gives bibliography).

³ v. Gorup-Besanez, *Ber. d. d. chem. Ges.*, vii., 146, 1874.

⁴ v. Gorup-Besanez, *Ber. d. d. chem. Ges.*, vii., 569, 1478.

⁵ *id.*, *Ber. d. d. chem. Ges.*, viii., 1510. Cf. *Sitzb. d. Erlanger phys. med. Soc.* (8), xi., 1874.

⁶ Green, *Philos. Transact. Royal Soc.*, clxxviii., 1887, 39.

VAN DER HARST¹ succeeded in isolating a similar ferment from the cotyledons of germinating garden beans. POEHL² has found a ferment resembling pepsin in the leaves of certain dicotyledons. NEUMEISTER³ opposed the statements of KRAUCH,⁴ who had found no ferments at all, but he was unable to confirm their occurrence in ungerminated *vetches* and *lupines* (GREEN, *loc. cit.*). Apart from this, he frequently met with proteolytic ferments in plants. HANSEN⁵ did not find any enzyme in *vetches*. GREEN⁶ also found one in the germinating seeds of *Ricinus communis*, and DACCOMO and TOMMASI⁷ in *Anagallis arvensis*. Proteolytic ferments were found by FERMI and BUSCAGLIONI⁸ in numerous plants and parts of plants, also in roots, tubers, &c. According to SCHEURER-KESTNER,⁹ a flesh-dissolving ferment is formed in the baking of bread.

Papaïn.—It was discovered long ago that the fruit and milky sap of the *papaw-tree*, *Carica papaya*, contained a substance which had an energetic action upon flesh, rendering it flabby and soft. Hence it was used by the aborigines of the Antilles and Brazil as a culinary adjunct.

The oldest descriptions of it extant are those of GRIFFITH-HUGHES¹⁰ in 1750, and of BROWN¹¹ in 1756. Fuller investigations on the botanical character of *Carica papaya* are described by HOOKER¹² and by WIGHT.¹³ Then WITTMACK¹⁴ studied the plant and also investigated the ferment.

This proteolytic ferment from the vegetable kingdom, which resembles *trypsin*, was first prepared by MONCORVO¹⁵ from the sap of *Carica papaya*, and named *caricin* by him.

¹ Van der Harst, *Naturforscher*, xi., 108, 1878.

² Poehl, *Ueber das Vork. u. d. Bildg. von Peptonen*, &c., Diss. Dorpat, 1882. Cf. *Biol. Centralbl.*, iii., 252. *B. d. d. chem. Ges.*, xiv., 1355.

³ Neumeister, *Z. f. Biolog.*, xxx.

⁴ Krauch, *Landwirthsch. Versuchsstat.*, 1879, 78; 1882, 303.

⁵ Hansen, *Arb. a. d. botan. Inst. Würzburg*, iii., 281.

⁶ Green, *Proc. Royal Soc.*, xlviii., 370.

⁷ Quoted by Green, *Ann. of Bot.*, vii., 112. Cf. *Maly's Jb.*, 1892.

⁸ Fermi and Buscaglioni, *C. f. Bakt. (II.)*, v., 125, 1899.

⁹ Scheurer-Kestner, *Comptes Rend.*, xc., 369.

¹⁰ * Hughes, *Natural History of Barbadoes*, 1750, vii., 181.

¹¹ * Brown, *Civil and Natural History of Jamaica*, 1756, 160.

¹² * Hooker, *Botan. Magazine*, New Series, iii., 2898.

¹³ * Wight, *Illustr. of Ind. Bot.*, ii., 34, 1850 (Nos. 10-13, quoted from by Wittmack, *vide infra*).

¹⁴ Wittmack, *Sitz. Ber. d. Ges. Naturforscher Freunde Berlin*, 1878, 40, gives all the older literature about *Carica papaya* and its action in general.

¹⁵ * Moncorvo, *Journal de Thérapie*, vii., 6, 1880.

BECKHOLT¹ gave an accurate account of the plant and its seeds, and also referred to the capacity for dissolving albumin possessed by the milky sap; he attempted to isolate this ferment, and gave it the name of *papayotin*. WITTMACK (*loc. cit.*) also tested the chemical activity of the fruit and sap.

WURTZ² was the first to examine it more thoroughly. He found it also in the leaves and stems of the plant, and gave it the name of *papaïn*, which, in addition to *papayotin*, is generally used at the present day. WURTZ³ precipitated it by means of alcohol from an aqueous extract of the sap, purified it by further treatment, and examined it more closely.

He regarded it as a substance of an albuminous nature. It is soluble in water, forming a neutral solution, which is non-diffusible, and becomes turbid on boiling. *Mercuric chloride* gives a turbidity in the cold and a precipitate on boiling. *Lead* gives a turbidity soluble in excess, and strong mineral acid precipitates also soluble in excess. Precipitates are produced by platinum chloride, tannin, and acetic acid with potassium ferrocyanide. It gives results similar to those obtained in the elementary analysis of albuminous substances, and contains sulphur.

According to MARTIN,⁴ commercial *papaïn* is not completely soluble in distilled water.

MARTIN (*loc. cit.*) confirmed, in the main, the reactions described by Wurtz. His aqueous extract contained an albumin, a globulin, and two albumoses. The ferment was attached to one of these, and MARTIN⁵ was unable to separate it therefrom.

He obtained the latter albumose by extraction with glycerin and precipitation with sodium and magnesium sulphates, or with a mixture of ether and alcohol. He named it α -*phytalalbumose*. It was identical with "vegetable peptone," Vine's hemi-albumose. It resembled Kühne's *proto-albumoses*, whilst the β -*phytalalbumose* was more akin to the hetero-albumoses. The globulin was related to myosin and paraglobulin.

HARLAY⁶ studied the influence of heat upon *papaïn*. He found that when dried it could resist heating to 100° C.; but that in solution it was weakened at 75° C. and destroyed at 82.5° C.

As regards the action of *papaya juice*, the first experiments were made by ROY,⁷ who found that it dissolved albuminous

¹ Beckholt, *Zeitschr. d. allg. österr. Apothekervereins*, xvii., 361, 373; *Pharmaceutical Journal*, 3rd Series, x., 343, 383.

² Wurtz and Bouchut, *Comptes Rend.*, lxxxix., 425, 1879.

³ Wurtz, *Comptes Rend.*, xc., 1379, 1880.

⁴ Martin, *Journ. of Physiol.*, v., 313, 1884.

⁵ Martin, *Brit. Med. Journ.*, 1885, 50; *Journ. of Physiol.*, vi., 336.

⁶ Harlay, *Journ. Pharm. Chem.* [6], xl., 268; *Chem. Centralbl.*, 1900, i., 918.

⁷ Roy, *Glasgow Med. Journ.*, 1874, quoted by Martin, *loc. cit.*

substances, though he did not describe the process as digestion; similar experiments with the juice were afterwards made by ALBRECHT.¹

WURTZ² attributed to it the property of digesting fibrin in a neutral solution, in which process it first combined with the fibrin and then dissolved it; peptones and leucine, he stated, were produced, but no tyrosine. ROSSBACH³ asserted erroneously that papain acted as energetically in the cold as on heating, which was refuted by MARTIN. The latter confirmed the statement of BRUNTON and WYATT,⁴ which had been questioned by ALBRECHT and ROSSBACH—viz., that an acid reaction prevented the action of the ferment. MARTIN⁵ used dried coagulated egg-albumin in addition to fibrin.

The ferment also reacts in a weak alkaline solution, the best being 0.25 per cent. solution of sodium hydroxide (MARTIN, *loc. cit.*).

WEEG⁶ found that the action took place best in neutral solution, and that it was checked by hydrochloric acid and by alkalinity; on the other hand, HIRSCHLER⁷ and SITTMANN⁸ found that dilute acids had a stimulating influence and alkalies a retarding effect, whilst CHITTENDEN⁹ obtained the same results in neutral, faintly acid, and faintly alkaline solutions. HIRSCH,¹⁰ again, found that the presence of hydrochloric acid, up to 0.2 per cent., had a beneficial effect. In such a solution fibrin was dissolved as rapidly as in a solution of pepsin and hydrochloric acid. The fermentation ceased in a 1 per cent. solution of sodium hydroxide.

In the digestion with papain a globulin is formed first, and this is then peptonised. Peptones and leucine can be detected in the liquid dialysing through a membrane; only a slight amount of tyrosine is produced.

The fermentation is checked by hydrocyanic acid, but only to a very slight extent by thymol.

¹ Albrecht, *Corresp. Bl. f. Schweizer Aerzte*, x., 680, 712, 1880; Schmidt's *Jahrb.*, exc., 4.

² Wurtz, *C. R.*, *loc. cit.*; also *C. R.*, xci., 787.

³ Rossbach, *Z. f. klin. Med.*, 1883, 527.

⁴ Brunton and Wyatt, *Practitioner*, 1880, 301, quoted by Martin.

⁵ Martin, *Journ. of Physiol.*, v., 220.

⁶ Weeg, *Ueber Papain*, Diss. Bonn, 1885.

⁷ Hirschler, *Ungar. Arch. f. Med.*, i., 341; *Maly's Jb.*, 1892, 19.

⁸ Sittmann, *Munch. med. Woch.*, 1893, 548.

⁹ Chittenden, *Transact. of the Connecticut Acad. of Arts and Sciences*, 1892, ix. Quoted in *Amer. Journ. of Med. Science*, 1893, 452.

¹⁰ Hirsch, *Therap. Monatsh.*, 1894, 609.

MARTIN¹ subsequently subjected the proteids of the juice itself to the digestive process, as had also been previously done by Wurtz. Globulin and albumin were first converted into β -phytalbunose, and then into bodies of a peptone character, which were also formed from the α -phytalbunose. Finally, leucine and tyrosine were produced.

CHITTENDEN² has made a quantitative estimation of the proportions of deutero-albumose and peptones in the digestion products. He finds that when a large quantity of papaya is present the amount of peptones is relatively increased.

Papaïn does not attack living protoplasm; it is completely innocuous (?); on subcutaneous injection it produces slight local symptoms (ROSSBACH³); on the other hand, it is said to destroy intestinal worms (TUSSAC⁴).

Therapeutically papaïn and similar preparations from the fruit of the *Carica papaya* (e.g., Reuss's papaïn) have been employed, on the one hand, to dissolve diphtheritic membranes,⁵ and, on the other, as an aid to digestion—e.g., when there is a deficiency of hydrochloric acid in the stomach, but with doubtful results (*inter alios*, notably, SITTMANN,⁶ HIRSCH,⁷ OSSWALD,⁸ GROTE⁹).

Papaya is also used on a large scale in the artificial peptonising of flesh, as, for instance, in the manufacture of Antweiler's¹⁰ and Cibil's¹¹ flesh peptones.

WITTMACK¹² and BOUCHUT¹³ have prepared from the juice of the fig tree, *Ficus carica* and *F. macrocarpa*, a ferment similar to papaïn, and CHITTENDEN¹⁴ has obtained from the juice of the pine-apple a proteolytic ferment more closely resembling pepsin, to which he has given the name of *bromelin*. HANSEN¹⁵ has investigated more fully the pepsin-resembling ferment of *Ficus carica*, and has prepared by its action *hemi*- and *anti*-albumose. In other milky saps—e.g., those of *Ficus elastica*, *Chelidonium*, and *Euphorbiaceæ*—no proteolytic enzymes were found by Hansen. GREEN¹⁶ discovered one in the fruit of *Cucumis uti-*

¹ Martin, *Journ. of Physiol.*, vi., 355.

² Chittenden, *Amer. Journ. of Physiol.*, i., 634, 1898.

³ Rossbach, *Z. f. klin. Med.*, 1883, 527.

⁴ Tussac, *Flore médic. des Antilles*, iii., quoted by Wittmack, *loc. cit.*

⁵ Rossbach, *Berl. klin. Woch.*, 1881, 133.

⁶ Sittmann, *Münch. med. Woch.*, 1893, 548.

⁷ Hirsch, *Therap. Monatsh.*, 1894, 609.

⁸ Osswald, *Münch. med. Woch.*, 1894, 665.

⁹ Grote, *Deutsche med. Woch.*, 1896, 474.

¹⁰ J. Munk, *Ther. Monatsh.*, 1888, 276.

¹¹ Rosenheim, *Krankh. d. Speiseröhre und d. Magens*, 1891, 134.

¹² Wittmack, *Vers. d. Naturf. u. Aerzte*, 1879, 222.

¹³ Bouchut, *Compt Rend.*, xci., 67, 1880.

¹⁴ Chittenden, *Journ. d. Physiol.*, xv., 249.

¹⁵ Hansen, *Arb. a. d. bot. Inst. Würzburg*, iii., 268.

¹⁶ Green, *Ann. of Botany*, vi., 95, 1892.

lissimus, both in the juice and in the pericarp. It works best in weakly alkaline media, and is thus similar to trypsin.

The so-called CARNIVOROUS PLANTS¹ approximate still more closely in this respect to the metabolism of animals.

They possess the power of utilising animal albumin, and thus it was to be expected that we should be able to obtain proteolytic enzymes from them.

The first publication on the albumin-digesting power of the glands of *Nepenthes* was made by HOOKER,² who was inclined, however, to attribute the activity less to secretion than to other factors, principally bacteria. DARWIN³ described a similar phenomenon in *Dionæa*.

The first experiments with *Drosera rotundifolia* were made by REES and WILL.⁴ They prepared glycerin extracts of the leaves, and obtained a slightly acid liquid, which had a peptonising action—i.e., on the addition of dilute hydrochloric acid. Similar results were obtained by v. GORUP-BESANEZ⁵ with *Nepenthes*, and almost simultaneously by LAWSON TAIT⁶ and VINES⁷ with *Nepenthes hybridus* and *N. gracilis*, the former using the leaves and juice itself, and the latter glycerin extracts as well. On stimulating the glands, v. GORUP-BESANEZ found an acid juice with strong digestive powers; when at rest, this was neutral, and showed but little activity, but on the addition of dilute hydrochloric acid it became active. He detected peptones in the liquid. HANSEN,⁸ too, found a proteolytic enzyme in *Nepenthes*.

VINES has also shown that the existence of a *zymogen* is very probable. He discovered no ferment in *Sarracenia*.

Completely analogous results have also been obtained in the case of insectivorous plants.

CANBY⁹ observed that flesh was dissolved by *Dionæa muscipula*, which even effected the solution of a fairly large *myriapod*.

COHN¹⁰ made similar observations in the case of *Aldrovandia*

¹ On this point see the comprehensive work of Pfeffer, *Landwirthsch. Jahrbücher*, vi., 969, 1877.

² Hooker, Address, British Association, reported in *Nature*, x., 366.

³ Darwin, *Insectivorous Plants*, 2nd edition, 1875.

⁴ Rees and Will, *Botanische Zeitg.*, x., 29, 1875; cf., *Sitzb. d. Erlanger phys. med. Soc.*, 1875, viii., 13.

⁵ Gorup-Besanez, *Ber. d. d. chem. Ges.*, ix., 673.

⁶ Lawson Tait, *Nature*, 1875, xii., 251.

⁷ Vines, *Journ. of Anat. and Physiol.*, xi., 124.

⁸ Hansen, *Arch. a. d. botan. Inst. Würzburg*, iii., 265.

⁹ Canby, *Oesterr. botan. Ztschr.*, xix., 77; xxv., 287.

¹⁰ Cohn, *Beiträge z. Biol. d. Pflanzen*, i., Part 3, 71, 1875.

vesiculosa and *Utricularia vulgaris*, and CANBY¹ in the case of *Darlingtonia Californica*.

MORREN,² who worked on *Drosera* and *Pinguicula*, went deeply into this question in numerous researches. At first he was not convinced of the identity of this process with the phenomena of digestion, but afterwards was converted to this view, and then supported it very vigorously.

The accepted views on the pepsins of insectivorous plants (*Nepenthes*) were attacked by DUBOIS,³ who could not observe any action when air was excluded, but only when bacteria had access (naturally not a very remarkable result, since his extract was only "légèrement acide," and he added no more hydrochloric acid). They were next attacked by TISCHUTKIN,⁴ who introduced pellets of albumin into closed tubes, and on their spontaneous opening could not detect any digestive action, but rather attributed the action exclusively to *bacteria*. This again was opposed by GOEBEL,⁵ and by VINES,⁶ who in his later experiments eliminated the organised ferments by the addition of a few c.c. of 2 per cent. hydrocyanic acid, and under these conditions was able to confirm his former results. *Leucine* was found in the products of the digestion, but no true peptones.

The *Nepenthes* enzyme, in particular, thus appears, like papaïn, to occupy an *intermediate position* between pepsin and trypsin. In its action it more closely resembles trypsin, but acts in acid solution, and is also very resistant to the influence of alkalies (Vines, *loc. cit.*).

Proteolytic Ferments in Cryptogams.—Proteolytic ferments also occur in moulds. POEHL⁷ found them in *Penicillium*, and BOURQUELOT and HERISSEY⁸ in *Aspergillus niger*. In other moulds they discovered a ferment which dissolved *not* fibrin or albumin, but *casein*.⁹ MALFITANO¹⁰ found in *Aspergillus* an *enzyme capable of isolation*, which was active in an acid solution.

HJORT¹¹ found in fungi (*Agaricus*, &c.) a *tryptic* ferment which

¹ Canby, *Oesterr. botan. Ztg.*, xxv., 287.

² Morren and others, *Bull. de l'Acad. de Sciences d. Belgique*, 2nd series, xxxix., 870; xl., 6, 525, 1040; xlii., 1019.

³ Dubois, *Comptes Rend.*, cxi., 315, 1890.

⁴ Tischutkin, Abstract in *Bot. Centralbl.*, l., 304, 1892.

⁵ Goebel, *Pflanzenbiol. Schilderung*, ii., 173, 1893.

⁶ Vines, *Annals of Botany*, xi., 563, 1897; xii., 545, 1898.

⁷ Poehl, *Biolog. Centralbl.*, iii., 252.

⁸ Bourquelot, *Bull. de la Soc. de Mycol. de France*, ix., 230. (Reprint.)

⁹ Bourquelot and Herissey, *C. R.*, cxxvii., 666. *Bull. Soc. Mycol.*, xv., 1899. (Reprint.)

¹⁰ Malfitano, *Annal. Inst. Pasteur*, xiii., 60, 1900.

¹¹ Hjort, *Centralbl. f. Physiol.*, x., 192, 1896.

worked best in a neutral solution and produced leucine, tyrosine, and tryptophan.

A proteolytic ferment was discovered by KRUKENBERG¹ in *Fuligo septica*.

According to GERET and HAHN,² the *endotrypsin* of yeast represents a new type of digestive ferment. It is only active in a slightly acid solution; on the other hand, its products resemble those of tryptic digestion. Albumoses are only formed to a very slight extent, and *no peptones at all*. At the end of the reaction 30 per cent. of the nitrogen is in the form of bases and 70 per cent. in the form of amido-acids. Oxygen, neutral salts, and dilute acids have a stimulating influence, whilst mercuric chloride, phenol, and concentrated solutions of glycerin and cane sugar are injurious, as is also concentration of the expressed yeast extract. The enzyme is not dialyzable; it gives neither the biuret nor Millon's reaction. The optimum temperature for its action is about 40° to 45° C., and it is destroyed at 60° C. At 37° C. it remains active for nine to fifteen days.

From *bacteria*, too, numerous proteolytic ferments have been isolated,³ some of which pass directly into the culture medium in an analogous manner to the *toxines* of pathogenic micro-organisms, and can be isolated by filtration through a porcelain filter, whilst others are firmly attached and can only be obtained by killing the bacteria (*e.g.*, by heat) in an analogous manner to yeast invertase. Thus they have been obtained from *anthrax* (HANKIN⁴), Koch's cholera vibrio (BITTER⁵), and other vibrios (MACFADYEN⁶), putrefactive bacteria (HÜFNER⁷), and others. BRUNTON and MACFADYEN⁸ have succeeded in isolating from bacteria *which liquefied gelatin* proteolytic enzymes, the action of which was checked by acids. WOOD⁹ prepared from different bacteria enzymes which were somewhat different, especially in their behaviour towards acids. VIGNAL³ succeeded in isolating the most opposite kinds of enzymes from *B. mesentericus vulgatus*.

According to LIBORIUS,¹⁰ no enzymes are separated if oxygen be excluded. FERMI,¹¹ who has made a thorough investigation

¹ Krukenberg, *Unters. phys. Inst. Heidelberg*, ii., 273.

² Geret and Hahn, *Z. f. Biol.*, 1900, xl., 117.

³ The literature is given by Flügge, *Micro-organismen*, 1896, 207.

⁴ Quoted from Green, *Ann. of Bot.*, vii.

⁵ Bitter, *Arch. f. Hygiene*, v., 245, 1886.

⁶ Macfadyen, *Journ. of Anat. and Physiol.*, xxvi., 409, 1892.

⁷ Hüfner, *Journ. pr. Ch.*, N.S., v., 872, 1872.

⁸ Brunton and Macfadyen, *Proc. Roy. Soc.*, xlv., 542, 1890.

⁹ Wood, *Labor. Reports, Roy. Coll. Phys. Edinburgh*, ii. Quoted from Green, *loc. cit.*

¹⁰ Liborius, *Zeitschr. f. Hyg.*, i., 115.

¹¹ Fermi, *Arch. f. Hyg.*, xiv., 1. Fermi and Pampersi, *Maly's Jb.*, 1897, 827.

of the tryptic bacterial ferments, states that they have only a solvent action on albumin, and do not peptonise it. He also obtained them from culture-media *free from albumin*. According to his results, the ferments of the different bacteria show the greatest variations in almost every respect. Those of the vibrios are the most resistant—*e.g.*, even towards more elevated temperatures. As regards their activity, these enzymes, as a rule, offer a greater resistance to poisons; according to Wood,¹ the cholera vibrio forms an exception, its enzyme being destroyed more rapidly by carbolic acid than the vibrio itself.

Although the vegetable ferments, which we have just described, are secretion-products of the cells and can be obtained from them without violent treatment, there are also proteolytic ferments in the lower plants, which, like Buchner's *zymase* (*q.v.*), are, as a rule, only active in the interior of the living cell, and can only be isolated from it by the same violent measures.

SALKOWSKI² was the first to state that *yeast*, when protected from putrefaction, digested itself.

HAHN³ next made the observation that the liquid expressed from yeast, after the addition of chloroform, possessed the power of dissolving phenol-gelatin. He then further studied this question in collaboration with GERET,⁴ and found that the liquids expressed from various yeasts contained proteolytic ferments. These produced *leucine* and *tyrosine* very rapidly, and also nucleic bases, phosphoric acid being split off; but, on the other hand, there were no true *peptones* formed. When albumoses were added, they rapidly underwent further decomposition.

Hydrocyanic acid, which, in very minute quantities, checks the activity of organised ferments, had no appreciable influence.

According to GERET and HAHN,⁵ exactly similar enzymes are present in the expressed liquids from the *bacilli of tuberculosis*, *typhus bacilli*, and *Sarcina rosea*, as also from *germinating lupine seeds*.

A similar proteolytic ferment was obtained by EMMERICH and Löw⁶ from *pyocyaneus* cultivations, which, when left to themselves, gradually died and liquefied. They named it *pyocyanase*. It possessed the properties of an enzyme, and dissolved fibrin and egg-albumin.

¹ Wood, *Labor. Reports*, Roy. Coll. Phys. Edinburgh, ii. Quoted from Green, *loc. cit.*

² Salkowski, *Zeitschr. f. klin. Med.*, xvii., Supplem. Z. *physiol. Ch.*, xiii., 506.

³ Hahn, *Ber. d. d. chem. Ges.*, xxxi., 200, 1898.

⁴ Geret and Hahn, *ibid.*, xxxi., 202, 2335.

⁵ Geret and Hahn, *ibid.*, xxxi., 2335.

⁶ Emmerich and Löw, *Zeitsch. f. Hygiene*, 1899, 1.

CHAPTER XIII.

RENNET (CHYMOSIN).

THE fact that the mucous membrane of the fourth or true stomach of the *calf* has the power of causing milk to coagulate has long been known,¹ and even in the remote past its practical results had been applied in the manufacture of cheese. BERZELIUS was the first to point out that the process was independent of the *formation of lactic acid*, to the influence of which the coagulation had at first been attributed. LIEBIG assumed that in the formation of lactic acid the alkali was taken into combination, and that thus the casein was precipitated; this view, however, was refuted by SELMI,² who showed that milk could also coagulate in an alkaline solution. The scientific discovery of the action of rennet was made by HEINTZ,³ who, in opposition to the older view, which regarded the rennet-coagulation as an action of *pepsin* or the gastric acid, or as connected with the *production of lactic acid* (SOXHLET⁴), showed that the mucous membrane of the stomach caused milk to coagulate both in acid and alkaline solution.

HAMMARSTÉN⁵ and A. SCHMIDT⁶ next proved that this coagulation is brought about by means of a ferment, to which he gave the name of *lab ferment* or *chymosin* (anglicé *rennet*). He showed the difference between this true rennet-coagulation and acid-coagulation, and proved conclusively by his experiments with solutions of casein, free from lactose, that the formation of lactic acid had no connection with the rennet coagulation. The coagulation was recognised as *fermentative action* by the fact that the whey poured off from cheese had the power of again causing coagulation.

¹ A very interesting historical review from the earliest times is given by Peters in his *Diss. on Rennet*, Rostock, 1894.

² Selmi, *J. Pharm. et Chim.* [3], ix., 265, 1846.

³ Heintz, *J. f. pr. Ch.*, N.S., vi., 374, 1872.

⁴ Soxhlet, *J. f. pr. Ch.*, N.S., vi., 1.

⁵ Hammarstén, abstract in *Maly's Jb.*, 1872, 118; *ibid.*, 1874, 135; 1877, 158.

⁶ A. Schmidt, *Beiträge z. Kenntn. d. Milch*, Dorpat, 1871.

Occurrence of Rennet.—The chief place for the normal production of rennet is the *mucous membrane of the stomach*, which contains it either in the free state (as in the calf and sheep) or more frequently as an inactive *zymogen*, which is transformed by acids into the active form. HAMMARSTÉN found it as a zymogen in the stomachs of *all* the animals which he examined for it.

It is also never wanting in sucklings (SZYDLAWSKI¹).

According to a recently-published research by BANG the *rennet* of man and of the pig differs so essentially from the ordinary rennet that he regards it as a special ferment and gives it the name of *parachymosin* (*vide infra*). Rennet is produced more abundantly in the fundus than in the pylorus; probably the *chief cells* and the granules contained in them are the source of production of the *rennet zymogen* as well as of the pepsinogen (GRÜTZNER²).

The *free enzyme* is produced very sparingly in the mucous lining of the stomach. In hunger it is, like the other ferments, present in greater abundance (LÖRCHER, *loc. cit.*). It is absent in severe diseases of the stomach—*e.g.*, *gastritis* and *carcinoma* (BOAS,³ JOHNSON,⁴ JOHANNESSEN⁵), but it is also found outside the stomach—*e.g.*, in the small intestine (BAGINSKI);⁶ it was found in the urine by HOLOVTSCHINER⁷ *inter alios*, whilst other observers did not find it there, and its occurrence was found by BOAS⁸ to be very irregular. EDMUNDS⁹ discovered rennet in the most diverse organs, and also in *dried testicle*.

The pancreatic juice has also an influence on milk, though of another kind (*vide Trypsin*).

Preparation of the Ferment.—To obtain rennet from the mucous membrane of the stomach, the latter is first treated for 24 hours at the ordinary temperature with hydrochloric acid of strength 0.1 to 0.2 per cent. in order to transform the zymogen into the enzyme. After filtering and carefully purifying this extract its coagulating power may then be tested.

Instead of this method a glycerin extract (HAMMARSTÉN) or a saturated aqueous solution of salicylic acid is employed (ERLENMEYER¹⁰), also a solution of common salt, &c.

¹ Szydlawski, *Prag. med. Woch.*, 1892, 365; *c.f.*, however, Schumburg, *Virch. A.*, xcvii., 260, 1884.

² Grützner, *Pflüg. A.*, xvi., 119. ³ Boas, *C. med. Wiss.*, 1887, 417.

⁴ Johnson, *Z. klin. Med.*, xiv., 240, 1888.

⁵ Johannesson, *Z. klin. Med.*, xvii., 204, 1890.

⁶ Baginski, *Z. f. physiol. Ch.*, vii., 209, 1882.

⁷ Holovtschiner, *Virch. A.*, civ., 42, 1886.

⁸ Boas, *Z. klin. Med.*, xiv., 249, 1888.

⁹ Edmunds, *Journal of Physiol.*, xix., 466, 1895.

¹⁰ Erlenmeyer, *Sitzb. Münch. Acad.*, 1875, 82.

By precipitation of these extracts with alcohol, an impure precipitate is obtained, which is active when re-dissolved in water.

LÖRCHER¹ employs a *glycerin* or, better, an *acid extract* of the dried mucous membrane. The acid extracts are the more active, the glycerin extracts the more stable. To separate it from the pepsin HAMMARSTÉN employed the following method:—

The hydrochloric acid infusion of the stomach was neutralised by shaking it with magnesium carbonate, and the *pepsin* then precipitated by the addition of a little lead acetate. The filtrate, which no longer acted upon fibrin, was again treated with ammonia and lead acetate, the precipitate containing the rennet decomposed with very dilute sulphuric acid, and the ferment isolated from the filtrate by means of *cholesterin* in the same way as in Brücke's method of separating pepsin.

Properties of Rennet.—It gives none of the ordinary proteid reactions, except that of forming a precipitate with lead acetate. According to MAYER,² however, it is precipitated by the salts of other heavy metals, though not always quantitatively.

It does not diffuse through animal membranes, and only with difficulty through porous porcelain.

It is destroyed—

By *alcohol* slowly, the rate increasing with the proportion of alcohol in the solution.

By *trypsin* and *putrefactive bacteria* (BAGINSKI³).

By *bile* and the sodium salt of choleic acid (BOAS, *loc. cit.*).

According to HAMMARSTÉN, gastric juice destroys *calves' rennet* after a digestion of 24 to 48 hours, but, on the other hand, according to THUNBERG, the rennet contained in *commercial pepsin* remains unchanged and active for several days if it is subsequently neutralised with calcium carbonate, and not with *alkalies*. BANG⁴ asserts that another ferment, *parachymosin*, is present here (*vide infra*). Its behaviour at high temperatures varies according to the reaction.

Whilst it is stable in a neutral solution up to 70° C., in dilute acid solution it is destroyed immediately at 63° C., and in about 40 hours at 40° C., and particularly rapidly in distilled water (CAMUS and GLEY⁵); by virtue of the last property solutions of pepsin can be freed from rennet, since pepsin is not affected in this way. It resists dry heat well, and is also more stable in *glycerin* (LÖRCHER, *loc. cit.*, 175). On the other hand, it remains

¹ Lörcher, *Pflüg. A.*, lxi., 141, 1898.

² Mayer, *Landw. Versuchsst.*, xxvii., 247, 1881.

³ Baginski, *Z. f. physiol. Chem.*, vii., 209, 1882.

⁴ Bang, *Pflüg. A.*, lxxix., 425, 1900.

⁵ Camus and Gley, *C. R.*, cxxv., 256.

uninjured at 0° C., especially in solutions containing a large amount of *lactic acid* (CAMUS and GLEY¹), but first *becomes active* at 10° C. and upwards.

It is very sensitive to the action of alkalies and alkali carbonates; it is destroyed even by a 0·025 per cent. solution of sodium hydroxide and a 1 per cent. solution of sodium carbonate (LANGLEY²). Light, too, injures the ferment (MAYER).

The Zymogen of Rennet.—The first assumption of a *pro-lab* was made by HAMMARSTÉN.³ GRÜTZNER⁴ established the existence of this zymogen.

It is secreted exclusively by the fundus glands, *like pepsinogen*, and is first converted into the ferment by the acid of the stomach, or in the artificial extraction with acids. Almost all acids can be used for the purpose. The best are hydrochloric and sulphuric acids, and the weakest acetic acid (LÖRCHER⁵); in the stomach, in the absence of hydrochloric acid, lactic acid (JOHANNESSEN⁶) and other organic acids (BOAS⁷) can also serve as a zymoplastic agent. It offers a greater resistance to the action both of alkalies and of heat than the ferment itself, as is also the case with pepsinogen (BOAS,⁷ KLEMPERER⁸).

Calcium chloride, according to Boas, endows the *pro-lab* with the power of coagulating milk; this, however, is contradicted by LÖRCHER.

The formation of the *ferment* from the zymogen is entirely dependent on the production of free acid, so that in the absence of the latter from the gastric juice the free ferment is invariably wanting, whilst the zymogen is present.

Estimation of the Activity.—As a rule, the activity of the ferment, or the amount of active ferment present, is measured by the time which a given quantity requires to effect the coagulation of a given quantity of milk, since in the case of rennet the time occupied by the action is almost inversely proportional to the amount of ferment.

MORGENROTH,⁹ instead, determines the smallest quantity of a definite solution of ferment, which coagulates the same quantity of milk under the same conditions. He keeps the

¹ Camus and Gley, *C. R.*, cxxv., 256.

² Langley, *Journ. of Physiol.*, iii., 259, 1883.

³ Hammarstén, *Lehrbuch. d. phys. Ch.*, 1896, 154.

⁴ Grützner, *Pflüg. A.*, xvi., 118, 1878.

⁵ Lörcher, *Pflüg. A.*, lxix., 183, 1898.

⁶ Johannesson, *Z. f. klin. Med.*, xiv., 256, 1888.

⁷ Boas, *Z. f. klin. Med.*, xiv., 256, 1888.

⁸ Klemperer, *Z. f. klin. Med.*, xiv., 282, 1888.

⁹ Morgenroth, *C.f. Bakt.*, xxvi., 349, 1899.

mixture of milk and ferment overnight at 0° to 8° C. ; no coagulation results from this. He then heats it rapidly to 32° C., and adds more of the ferment solution until coagulation occurs. In this way he was able to determine the requisite minimum and to take this as the unit.

Action of Rennet.—The externally recognisable action of the enzymes is that the milk is coagulated in the presence of calcium salts. In this process, according to MAYER (*loc. cit.*) there is a positive manifestation of heat.

The casein of the milk is, in HAMMARSTÉN'S opinion, decomposed into two other proteids, an *albumose* free from phosphorus left in the whey, and *paracasein*¹ containing phosphorus, which represents the *cheese*. Hammarstén obtained the same decomposition by heating a solution of *casein* in a tube to 130° C. The albumose was more closely examined by KÖSTER.² PETERS asserts that *rennet* also coagulates other albuminous substances—*e.g.*, alkali albuminate from egg-albumin—but this is denied *inter alios* by EDMUNDS,³ who observed no coagulation, but only a partial precipitation produced by the calcium salts. Casein and paracasein differ from one another in the following particulars:—*Casein* is precipitated *unaltered* from the milk by common salt, magnesium sulphate, &c., and also by dilute acids, and can be dissolved again. As soon as it has been precipitated by *rennet*, however, it changes its properties. It becomes insoluble in water; *paracasein* is precipitated from its solution in lime water by phosphoric acid, while *casein* is not. It dissolves without alteration in a solution of ammonium oxalate (EDMUNDS³). HAMMARSTÉN purified paracasein by dissolving it in very dilute ammonium hydroxide and precipitating it with acetic acid. Its properties vary slightly according to the method of preparation. But above all, this solution of *paracasein* cannot be re-precipitated by *rennet*.

This statement has been controverted by PETERS (*loc. cit.*), who asserted that a solution of paracasein in very weak lime water could invariably be made to coagulate again at will.

HAMMARSTÉN⁴ refuted this assertion in a very careful research, and attributed it to the fact that Peters had worked with an extract of rennet containing a large proportion of salts, so that the paracasein was *precipitated* by the sodium chloride, but not

¹ This is Hammarstén's terminology, which it is best to follow. Others—*e.g.*, Peters (*Untersuch. üb. das. Lab*, Diss. Rostock, 1894)—term Hammarstén's casein *caseinogen* and his paracasein *casein*.

² Köster, *Maly's Jb.*, xi., 14, 1881.

³ Edmunds, *Journ. of Physiol.*, xix., 466, 1895.

⁴ Hammarstén, *Z. physiol. Ch.*, xxii., 130, 1896-7.

coagulated by the rennet. Soluble calcium salts also form a compound with paracasein which is less soluble on heating than in the cold (RINGER¹). The behaviour of calcium-paracasein solution towards common salt is thus also a characteristic which distinguishes it from casein, since the latter is *not* thus precipitated by dilute sodium chloride solutions. On heating the solution there is frequently a deposition of some paracasein, which appears to be changed by heat.

In the coagulation of milk the deposition of the paracasein is only a *secondary* phenomenon.²

Thus, when a solution of casein in chalk water is prepared, care being taken to avoid an excess of chalk (trituration of the casein in water with pure calcium carbonate)³ an approximately neutral solution of calcium casein is obtained. This is *not* coagulated by pure rennet. As soon, however, as a *soluble calcium salt* is added, coagulation occurs; the *paracasein* already formed, but still in solution, is deposited. The same result is obtained with sodium chloride free from calcium (HAMMARSTÉN), though only in greater degrees of concentration, and frequently first at the temperature of the body and upwards. The action of soluble calcium salts is thus not necessary for the formation of *paracasein*, but they or other salts are required only for the *precipitation* of the paracasein already formed, and they must thus recede from the important position assigned to them in the rennet coagulation since the investigations of SÖLDNER.⁴ He laid stress upon the importance of the *soluble* salts, but attributed no significance to the *calcium phosphate* retained in solution by the casein, in opposition to Hammarstén's earlier views, in which only the *calcium phosphate* was regarded as active.

ARTHUS and PAGÈS⁵ regard *cheese* as a calcium compound of *caseogen*, which, in its turn, is a decomposition-product of casein.

Although Hammarstén was unable to bring about a completely typical coagulation in solutions of pure paracasein by means of sodium chloride, but only a precipitation, yet he succeeded in doing this with *dialysed milk* in the absence of *precipitable* soluble calcium salts.

According to ARTHUS⁵ and PETERS,⁶ calcium salts can also be replaced

¹ Ringer, *Journ. of Physiol.*, xi., 464.

² Cf. Arthus and Pagès, *Arch. d. Phys.* [5], ii., 330, 540, 1890.

³ Hammarstén at first prepared this "artificial milk" by dissolving the casein and neutralising the solution with phosphoric acid. This solution was capable of coagulation, in which phenomenon H. assigned an important part to the calcium phosphate. LUNDBERG (*Maly's Jb.*, 1876, 11) showed that an addition of *oxalic acid* to the casein solution prevented the coagulation, as also did sulphuric acid in *baryta* solutions of casein.

⁴ Söldner, *Landw. Versuchsst.*, xxxv., 357.

⁵ Arthus and Pagès, *loc. cit.*, 540.

⁶ Peters, *loc. cit.*, 26.

by barium or strontium salts. EUGLING¹ ascribes to casein the structure of a tricalcium casein phosphate, and assumes that this is decomposed by rennet into a soluble calcium phosphate compound. This view has been opposed by SÖLDNER in the research referred to above, in which he denies the existence of such a compound of casein with calcium phosphate. DE JAGER² is inclined to attribute to the soluble calcium salts the important function of converting other compounds of casein (*e.g.*, sodium casein) into the calcium salt which readily coagulates. COURANT³ concludes that only one of the casein calcium phosphate salts (*viz.*, the dicalcium casein phosphate which is acid to phenolphthalein and alkaline to lacmoid) is coagulable, and that neutral calcium tri-phosphate, which is formed in the presence of soluble calcium salts and phosphoric acid, must also be present. He attributes the inferior coagulating power of *human milk* to its greater alkalinity. This is supported by the observations of RAUDNITZ⁴ and SZYDLAWSKI,⁵ that it too gives a typical coagulation after being rendered slightly acid.

Activity of the Ferment.—HAMMARSTÉN found that *rennet*, the product being regarded as a pure ferment, was able to transform from 400,000 to 800,000 times its quantity of casein.

According to MAYER,⁶ the time occupied by the action of rennet is inversely proportional to the quantity. PETERS (*loc. cit.*) confirmed this, but found that when a sufficient quantity of ferment was present, any further addition had no accelerating influence, which, indeed, is also the case with other ferments.

BENJAMIN⁷ confirmed the fact that the coagulation takes place most rapidly in faintly acid solution, more slowly in neutral solution, and worst of all in alkaline solution.

The rapidity of coagulation, according to his observations, is further reduced: by the milk being shaken with chloroform, on increasing dilution, and on dilution with chloroform water.

Contrary to the experience of EUGLING⁸ and SCHÄFFER,⁹ he was able to cause boiled milk to coagulate without further treatment; LÖRCHER,¹⁰ on the other hand, asserts that it coagulates more slowly.

Sterilised milk is *not* coagulated by *rennet*. According to PETERS (*loc. cit.*), the ferment is most active at the *temperature of the body*, and the same is asserted by MAYER,¹¹ who found that

¹ Eugling, *Landw. Versuchsst.*, xxxi., 391, 1885.

² De Jager, *Maly's Jb.*, 1897, 276.

³ Courant, *Reaction der Kuh- und Frauenmilch*, Diss. Breslau, 1891.

⁴ Raudnitz, *Prag. med. Woch.*, 1887, 24.

⁵ Szydlawski, *Prag. med. Woch.*, 1892, 365.

⁶ A. Mayer, *Landw. Versuchsst.*, xxvii., 247, 1882.

⁷ Benjamin, *Virch. A.*, cxlv., 30, 1896.

⁸ Eugling, *Landw. Versuchsst.*, xxxi., 391, 1885.

⁹ Schäffer, *Maly's Jb.*, 1887, 158.

¹⁰ Lörcher, *Pflüg. A.*, lxix., 141, 1898.

¹¹ A. Mayer, *Landw. Versuchsst.*, xxvii., 247, 1882.

its activity was only one-third as great at 25° C., and that its action ceased at as low as 45° C. Boas¹ found the optimum to be 35° to 40° C.; according to Lörcher it is active from 10° to 60° C., and at a lower temperature in the case of the frog.

Of salts, *ammonium sulphate* notably showed a restrictive influence, but other salts, too—carbonates, sulphates, and nitrates—acted injuriously. Sodium chloride up to 0.9 per cent. has a beneficial influence, and above that a restrictive one (MAYER, *loc. cit.*). Sodium fluoride and calcium oxalate prevent the action. Magnesium salts, as also those of zinc, aluminium, and cadmium have a stimulating effect (LÖRCHER²), as has also, in a special degree, calcium chloride (RINGER,³ Boas [*loc. cit.*]). The influence of acids has been investigated by PFLEIDERER.⁴ He found that hydrochloric acid had the most accelerating influence, and then nitric acid, lactic acid, acetic acid, sulphuric acid, and phosphoric acid. Boric acid is without influence (MAYER⁵).

Blood serum has a restrictive influence on its action (RÖDEN,⁶ BRIOT⁷).

Peptones retard its activity, especially when dissolved in an 8 per cent. solution of sodium chloride (GLEY,⁸ EDMUNDS,⁹ LOCKE¹⁰).

Potassium sulphocyanide has a prejudicial influence (WRÓBLEWSKI¹¹).

According to BENJAMIN, chloroform in small amount has at first a beneficial action, but afterwards, and in larger quantities, it has a restrictive influence.

FREUDENREICH¹² investigated the influence of various anti-septic agents on rennet. He found that its activity was greatly injured by thymol and by formaldehyde vapour; but that chloroform and formalin, in 0.5 to 1 per cent. solution, have little influence. *Alkaloids* have a favouring influence (PETERS).

The subcutaneous introduction of rennet in small quantities produces an *immunity* against the ferment, which points to the

¹ Boas, *Z. f. klin. Med.*, xiv., 249, 1888. Cf. Johnson, *Z. klin. Med.*, xiv., 243, 1888.

² Lörcher, *loc. cit.*

³ Ringer, *J. of Physiol.*, xi., 464.

⁴ Pfeleiderer, *Pflüg. A.*, lxvi., 605, 1897.

⁵ Mayer, *Enzymologie*, 49.

⁶ Röden, *Maly's Jb.*, xvii., 160, 1887.

⁷ Briot, *C. R.*, cxxviii., 1366, 1899.

⁸ Gley, *C. R. Soc. Biol.*, xlviii., 591, 1896.

⁹ Edmunds, *Journ. of Physiol.*, xix., 466, 1896.

¹⁰ Locke, *Journ. of Exper. Medic.*, ii., 493. Quoted from Lörcher.

¹¹ Wróblewski, *Ber. d. d. chem. Ges.*, xxviii., 1719, 1895.

¹² Freudenreich, *C. f. Bakt. (IL.)* iv., 309, 1898.

conclusion that an *anti-rennet* is present in the milk and serum of the immunised animals, and the addition of this to milk prevents its coagulation. MORGENROTH,¹ to whom we owe this interesting discovery, has made a quantitative examination of the rennet immune serum, and has found that the proportion of anti-toxine varies greatly in the different sera. The strongest immune serum obtained by him prevented coagulation when added to milk in the proportion of 2 per cent., the amount of ferment added being 1:20,000, whilst coagulation occurred when the amount of ferment was as 1:15,000. In the absence of anti-rennet, the coagulation took place after the addition of 1:3,000,000. Thus, in this case, after the addition of anti-rennet, *200 times more ferment* was required to effect the coagulation. We have discussed the theoretical significance of this discovery in the general part. Anti-rennet is very unstable. Morgenroth also attributes the above-mentioned *restrictive influence of different blood sera*, notably of that of horses' blood, *on coagulation*, to the normal presence of the anti-body.

Parachymosin.—As we have briefly pointed out above, it appears from the discoveries of BANG² as though there were two milk-coagulating ferments in the animal kingdom. Thus Bang found the rennet of man and of the pig to differ greatly from that of other animals, and particularly from the ordinary ferment of the stomach of the calf.

THUNBERG, by the way, had previously discovered that the rennet contained in commercial *pepsin* (from the stomachs of pigs) no longer coagulated milk after being digested for several days in dilute acid solution and then neutralised with alkali, though it did so when neutralised with calcium carbonate. BANG now found that *calves' rennet* behaved in a *completely similar manner* with either alkalising agent. From this he concluded that pepsin rennet was differently constituted to calves' rennet, and found this confirmed on further investigation. The ordinary *chymosin* differs from the new *parachymosin* in the following particulars:—

Parachymosin does not follow the law applying to rennet that the time of reaction is inversely proportional to the amount of ferment, but its activity decreases *very much more rapidly* as the degree of dilution increases, and becomes absolutely *nil* in very dilute solutions.

¹ Morgenroth, *C. f. Bakt.*, xxvi., 349, 1899.

² Bang, *Pflüg. A.*, lxxix., 425, 1900.

Calcium chloride has a much more pronounced accelerating influence upon *parachymosin* than on *chymosin*.

Parachymosin offers greater resistance to heat, and under certain conditions still remains active for some time at 75° C.

On the other hand, it is more sensitive than *chymosin* to the action of *alkalies*.

In mixtures of the ferments either the *chymosin* may be destroyed by heating, or the *parachymosin* by means of alkali; this fact, in particular, led BANG to the conclusion that there were *two ferments*.

Vegetable Rennet.—The property possessed by the juice of certain plants of causing milk to coagulate was known as early as the sixteenth century, notably in the case of *Galium verum*, which, according to GREEN,¹ is still in use at the present day for the coagulation of milk. There are also *Pinguicula vulgaris*, which, according to LINNÉ,² is used for this purpose in Lapland, and, according to Pfeiffer, in the Italian Alps, *Drosera* (DARWIN³), *Carica papaya* (MARTIN⁴), &c.

BAGINSKI⁵ discovered the ferment in artichokes, *Carica papaya*, and other plants.

It has been more closely investigated by LEA,⁶ who isolated it by means of glycerin or sodium chloride solution from the seeds of *Withania coagulans*, a member of the *Solanaceæ* which grows wild in Afghanistan and India. It possesses similar properties to animal rennet. It is of practical interest in that the Hindoos use no rennet of animal origin on religious grounds.

GREEN⁷ discovered it with others in the germinating seeds of *Ricinus communis*, where it is present as a *zymogen*, which is rendered active by means of dilute acids. It is combined with the *trypsin*.

It acts both in acid and alkaline solution.

The fruit of the "Naras" plant (*Acanthosicyos horrida*) of South Africa also contains a rennet, which is said to be soluble even in 60 per cent. alcohol (MARLOTH⁸); this is also the case with Chittenden's *bromelin*.⁹

¹ Green, *Annals of Botany*, vii., 112.

² Quoted by Green, *loc. cit.*

³ Darwin, *Insectivorous Plants*, 2nd Edition, 1875, 114.

⁴ Martin, *Journ. of Physiol.*, vi., 340.

⁵ Baginski. *Z. physiol. Ch.*, vii., 209, 1882.

⁶ Lea, *Proc. Roy. Soc.*, xxxvi., 55, Nov. 1883.

⁷ Green, *Proc. Roy. Soc.*, xlviii., 391, 1890.

⁸ Marloth, quoted from Green's abstract in *Nature*, 1888, 275.

⁹ Chittenden, *Journal of Physiol.*, xv., 249.

PETERS¹ also found rennet in numerous plants—figs, artichokes, wild madder, thistles, and also in papaya juice. ROSETTI² studied the milk-coagulating ferment of artichokes, and proposed for it the very indefinite name of *cynarase*.

Finally, it has been found in bacterial enzymes³—*e.g.*, those of *Bacillus amylobacter* (FITZ and HUEPPE⁴), *Bacillus mesentericus vulgatus* (VIGNAL⁵), *Bacillus prodigiosus* (not destroyed until after one hour's heating at 100° C. [?]) (GORINI⁶), and in cholera vibrios (FOKKER⁷). CONN⁸ has isolated a typical rennet from various bacteria, and KALISCHER⁹ has prepared it from the bacteria which occur in milk.

Pectase is an enzyme which effects the coagulation of vegetable substances containing pectin.

It was first observed by FRÉMY,¹⁰ who found a soluble form of the ferment in carrots and other roots, and an insoluble form in acid fruits. The fermentation proceeds without the admission of air and without an evolution of gas, the most favourable temperature being 30° C. BERTRAND and MALLÈVRE¹¹ then investigated it more closely. They found that a coagulation of the calcium salt of pectic acid occurred when the plant juices were allowed to stand. Boiling, or precipitation of the calcium prevented the coagulation, which, however, could also be induced by means of barium or strontium salts. The solution had to be neutral, since acids rapidly injured the ferment.

They found it to be widely distributed in many plants, and also in cryptogams. On the other hand, they were unable to discover Frémy's insoluble pectase in acid fruits.

The Fibrin Ferment.—This appears to be the proper place to allude to that hypothetical ferment, which according to a widespread view, plays an important part in the process of the coagulation of the blood. It is regarded as a *coagulating* ferment, like rennet. It has been assumed that by means of its enzymic activity one of the native proteids of the blood, *fibrinogen*, is *decomposed*, in an analogous manner to casein, into *fibrin* and a

¹ Peters, *Unters. üb. das Lab*, Diss. Rostock, 1894, 45.

² Rosetti, *Chem. Centralbl.*, 1899, i., 131.

³ Duclaux, *Comptes Rendus*, xci. Hueppe, *D. med. Woch.*, 1884, 777.

⁴ Fitz and Hueppe, quoted by de Bary, *Vorlesg. üb. Bacter.*, 1885.

⁵ Vignal, quoted from Green, *Ann. of Bot.*, vii., 120.

⁶ Gorini, *Hyg. Rdsch.*, 1893, 381.

⁷ Fokker, *D. med. Woch.*, 1151, 1892.

⁸ Conn, *C. f. Bakt.*, xii., 223.

⁹ Kalischer, *Arch. f. Hyg.*, xxxvii., 30; *Chem. Centr.*, 1900, i., 177.

¹⁰ Frémy, *Journ. d. Pharm.*, xxvi., 392.

¹¹ Bertrand and Mallèvre, *Comptes Rendus*, cxix., 1012; cxx., 110; cxxi., 726.

globulin. Moreover, the calcium salts are said to have great significance for the process. I have refrained from dealing more fully with this ferment here for two different reasons. In the first place, it is not certain that a "ferment" in our sense co-operates here; according to the views of many investigators the decompositions which here occur appear to be of a synthetic rather than of a fermentative character. And, indeed, the originator of the fibrin ferment, A. SCHMIDT, could not have intended to indicate a ferment in our sense in the active principle which brought about the *combination of* fibrinoplastic and fibrinogenous substances; for a combination of two substances *can never* be effected by a ferment. But, above all, it would not have been possible to give a full description of the fibrin ferment here without following up in all its ramifications the whole subject of the *coagulation of the blood*, which is so extremely complicated and, in spite of vigorous efforts, still so obscure, since only in this environment should we have been able to study more closely the nature and conditions of activity of the hypothetical ferment. And for this the present book did not appear suitable. Besides, the "fibrin ferment" is not sufficiently characterised as such for the whole subject of the coagulation of the blood to be discussed as an essentially *fermentative* process in a work on ferments.

CHAPTER XIV.

THE SACCHARIFYING FERMENTS.

UNDER this heading we can group together a number of enzymes, the activity of which is exercised upon the *carbohydrates*. They possess the power of transforming more complex substances of this group into simpler compounds.

Thus, from starch, maltose and dextrin are produced by means of *diastase*; from maltose, glucose by *maltase*; from cane sugar, glucose and fructose by the action of *invertase*, &c. This specific character, however, must be understood *cum grano salis*, since the ferments are also capable of decomposing certain other substances of a similar character. Their activity is directed towards *single substances* or small groups of closely-related substances.

The final products of the action of these different enzymes are invariably simple aldoses or ketoses, *glucose* being usually one of the decomposition products.

The process itself must be regarded as a simple *hydrolytic decomposition*, analogous to that effected by dilute acids.

The saccharifying ferments are very widely distributed throughout the animal and vegetable kingdoms, and play a very important part in the economy of the organisms, since from the complex non-assimilable carbohydrates they prepare the simple sugars, which the organism can then utilise for its vital requirements. Thus they fulfil the same function for the carbohydrates as the proteolytic ferments for the proteids. Although certain authorities uphold the view, especially as regards the *decomposition of starch*, that it is, partially at least, to be attributed to a direct activity of the cell, yet they also cannot deny the importance of the sugar-producing *enzymes* in the nourishment of the organism.

Nomenclature.—As no general agreement has as yet been attained with regard to the names applied to this group of ferments, we must, in the first place, come to an understanding as to the nomenclature to be used here.

In general, authorities now adhere firmly to the principle of describing these ferments by forming their name with the suffix "ase" from the name of the *substance* upon which they exercise their *special hydrolytic action*.

In accordance with this rule the starch-decomposing ferments must therefore be described as *amylase*. The historical name *diastase* has, however, become so firmly established that it cannot now be put aside.

We shall, therefore, retain the term *diastase* as the collective name for the *starch-decomposing enzymes*.

BEYERINCK¹ suggests the application of the term *amylase* as the collective name for all the ferments which take part in the conversion of starch into its final products. To this proposition I must object on two grounds. In the first place, we have here to deal not with a single ferment, or not even with a group of ferments acting in a uniform manner, which could be described by the name "amylase," but at best could only say "amylases;" but above all the *natural* classification is not to group together the ferments which *decompose starch*, but those which produce *sugars* from polysaccharides; *inulinase*, *cytase*, &c., come strictly under this heading. I have for this reason described them as *saccharifying ferments*, the only objection to be made to this being that the ferments which *decompose glucosides* also produce sugar; yet the latter group, again, is such a natural unit that they may well be differentiated as a separate class from the saccharifying ferments in the narrower sense. The *saccharifying* ferments therefore include, in addition to the enzymes which decompose starch, those which convert the other polysaccharides into simpler sugars. In accordance with the above-mentioned principle *inulinase*, *pectinase*, *carubinase*, *lactase*, *trehalase*, *meliobiase*, and *maltase* are correctly designated. On the other hand, the ferment which decomposes cellulose is not in an analogous manner termed *cellulase*, but *cytase* or *cytohydrolytic* ferment. In the case of the enzyme of cane sugars also, the historic name *invertin*, in its modern form of *invertase*, has become so naturalised that neither the name *sucrase*, frequently used for it by the French, nor that of *saccharase*, which would be the best in accordance with the principle, can be successfully substituted for it.

There is false construction, too, in the name *seminase* as applied to the enzyme which decomposes the *horn albumin* (*mannan* and *galactan*) of certain seeds, since *seminase* can be used with as much right for all the other enzymes of seeds.

¹ Beyerinck, *C. f. Bakt.* (II.), i., 221, 1895.

A particularly hopeless confusion has been produced in the matter of the connotation of *maltase*, by the fact that Wijsman and Beyerinck understand by maltase an enzyme which decomposes *starch* into erythrodextrin and maltose. In addition to this, another starch-decomposing enzyme is said to exist, which produces *achroodextrin* (according to Beyerinck, in addition to maltose) from starch. Wijsman has designated this *dextrinase*, and Beyerinck *granulase*. *Dextrinase* is obviously false, since it does not decompose dextrin but produces it, and *granulase* is an absolutely colourless word. Granting that the assumption of two enzymes instead of diastase is correct, it would have been most fitting to describe them *both* as *amylase* to signify their common substratum—*starch*; and to differentiate them by the pregnant titles of *erythroamylase* and *achrooamylase*. The ordinary *maltase* is described by many as *glucase*, an entirely incorrect terminology, since it does not decompose glucose but produces it; and this property, even apart from the above-mentioned principle, cannot be chosen as the basis of nomenclature, since *glucose* is produced by nearly all saccharifying enzymes.

Determination of the Activity.—In estimating the activity of saccharifying ferments, *three methods* are, in the main, employed.

The *first* is naturally the chemical examination of the *ferment mixture* after the completion of the reaction, in which an attempt is made to identify the resulting products as such or in the form of characteristic compounds, especially *hydrazones* and *osazones*; or in which conclusions as to the nature and amount of the sugars are drawn from determinations of the *reducing power* and optical rotation. A still more simple, though less exact, method of the same character is the use of distinctive colour reactions—*e.g.*, the iodine test, the *disappearance of starch*, or Moore's test, the *production of sugars*.

Although this exact chemical investigation is theoretically the ideal one, yet in practice it is beset with great difficulties, because the separation of the different carbohydrates from one another is very tedious and unreliable; and so we see many important questions in this domain still remaining unsolved, as, for example, the existence of *isomaltose*, although we have acquired in the *osazone test* a direct means of solving certain previously obscure problems—*e.g.*, the existence of *maltase*.

Hence, methods have been sought which would detect individual items in the course of the reaction, independently of a complete analysis, particularly the production of a distinctive carbohydrate characteristic of the activity of a given enzyme.

To this class belongs specially BEYERINCK's¹ *auxanographic method*. Beyerinck detects the existence of—*e.g.*, *glucose*, by inoculating the mixture under examination with mould-fungi, which only grow upon *glucose* (and on *fructose*, which, in this experiment, need not be taken into account). If they grow, *glucose* is present; but, if the culture remains sterile, it is absent. In this way it is possible, for example, to detect *glucose* in the presence of *maltose* by means of *Saccharomyces apiculatus*, which cannot attack *maltose*.

The third method has been employed, notably by WIJSMAN, to demonstrate his two starch-decomposing enzymes, and is based upon *diffusion*. Gelatin plates are impregnated with the substratum to be fermented (*starch*), and a few drops of the solution of the ferment placed upon one spot of a plate. The ferment then slowly diffuses into the starch-gelatin, where it completes its action. If, now, a colour reaction be used in which the original substance is coloured differently from the decomposition products, there is formed, round the point where the ferment was applied, a zone which is of a different colour to the remainder of the gelatin mass—*e.g.*, in the iodine test for starch it is colourless against the blue of the starch. If different ferments attacking the same substratum are present, and diffuse at a different rate, differential colorations of the various zones are also produced (see Wijzman's "*maltase*").

DIASTASE.

Under the name of *diastase*, *amylase*, or *amylolytic ferment*, is understood an enzyme, or rather several enzymes, of similar activity, which possess the power of producing *maltose* and *dextrins* from *starch* by means of a *hydrolytic process*.

Diastase is found in numerous organs and secretions of plants and animals; above all, in *malt*, in *cryptogams*, in *saliva*, in the *pancreas*, and in the *liver*. *Malt diastase* has been known for the longest time, and has been the most thoroughly investigated.

The scientific discovery of *diastase* was made in the year 1833 by PAYEN and PERSOZ,² who gave it its name; the conversion of starch into sugar had already been observed by IRVINE³ and by KIRCHHOFF,⁴ and almost simultaneously SAUSSURE⁵ had

¹ Beyerinck, *C. f. Bakt.* (IL.), i., 221, 1895.

² Payen and Persoz, *Ann. d. Ch. et Phys.*, liii., 78.

³ Irvine, quoted by Payen and Persoz, *loc. cit.*

⁴ Kirchhoff, *Schweigg. Journ.*, xiv., 389, 1815.

⁵ Saussure, *Poggendorff's Ann.*, xxxii., 194, 1834. For the history of *diastase*, see Dubrunfaut, *Z. ges. Brauw.*, 1880, 99.

studied the starch-dissolving substance and regarded it as in combination with the mucin of the paste. The most important facts had already been discovered by DUBRUNFAUT.¹ Payen and Persoz endeavoured to obtain a purer substance by precipitation with alcohol. Similar attempts were made by DUBRUNFAUT,² BARANETZKI,³ and DUQUESNEL.⁴ To obtain serviceable preparations of diastase, other methods of isolating enzymes were tried by COHNHEIM⁵ (who made use of Brücke's method of preparing pepsin, *q.v.*), and by ZULOWSKY,⁶ who shook the aqueous extract with ether, thereby obtaining a "gelatinous mass resembling frog-spawn," which he precipitated with ether, and subsequently employed glycerin extracts,⁷ which were precipitated with alcohol. Of the older methods, the best is that of LINTNER.⁸ He extracted for a longer time with 20 per cent. alcohol, precipitated directly or fractionated with absolute alcohol, and triturated the preparation with absolute alcohol and ether. The preparations were then repeatedly dissolved in water and reprecipitated. He invariably obtained preparations containing a large proportion of ash (about 10 per cent.), part of which could afterwards be eliminated by dialysis. About 5 per cent. of neutral (normal) calcium phosphate, however, was invariably left behind. Glycerin extracts yielded solutions of the ferments of only very slight activity. OSBORNE and CAMPBELL⁹ have obtained preparations of diastase by "salting out" with ammonium sulphate, and WRÓBLEWSKI,¹⁰ by extraction with weak alcohol and precipitation with stronger alcohol, separated preparations which, in order to obtain purer substances, he submitted to further processes of purification (*vide infra*).

GRÜSS¹¹ obtained diastatically-active glycerin solutions by leaving very thin sections of the germinating seeds of plants for a long period in glycerin, when the diastase slowly diffused into the glycerin, being fairly

¹ Dubrunfaut, "Mem. sur la Sacharification," *Soc. d'Agricult.*, Paris, 1823.

² Dubrunfaut, *Dingler's polyt. Journ.*, clxxxvii., 491, 1868.

³ Baranetzki, *Die stärkeumbildenden Fermente in d. Pflanzen*, Leipzig, 1878, 10.

⁴ Duquesnel, *Bull. d. Thérap.*, lxxxvii., 20.

⁵ Cohnheim, *Virch. A.*, 1863, xxviii., 241.

⁶ Zulowsky and König, *Wiener Acad. Sitzb., Math. Nat. Cl. (II.)*, lxxi., 453.

⁷ Zulowsky, *ibid.* (II.), lxxvii., 647.

⁸ Lintner, *J. pr. Ch.*, N.S., xxxiv., 378; xxxvi., 481, 1886-7.

⁹ Osborne and Campbell, *Journ. Amer. Chem. Soc.*, xviii. *Ber. d. d. chem. Ges.*, xxix. (4), 1159, 1896.

¹⁰ Wróblewski, *Z. physiol. Ch.*, xxiv., 173.

¹¹ Grüss, *Jahrbüch. f. wissensch. Botanik*, xxvi., 379.

free from tannins and maltose. REYCHLER¹ prepared an "artificial diastase" by heating wheat-paste with dilute acids to 30° to 40° C. In this case, we are doubtless dealing with a production of activity in the zymogen through the action of the dilute acids.

Properties of Diastase.—Very divergent views are held as to the nature of the enzyme.

Whilst many investigators (*e.g.*, LOEW,² BROWN and HERON,³ SCHWARZER,⁴ and OSBORNE⁵) regard diastase as a proteid, and some (*e.g.*, MULDER [*loc. cit.*], BARANETZKI [*loc. cit.*, 54], SEESEN and KRATZSCHMER,⁶ BIZIO,⁷ who obtained reducing sugars from glycogen through the action of "proteids," and others) attribute diastatic capacity to the proteids in general, BARTH,⁸ LINTNER,⁹ and HÜFNER¹⁰ consider that it differs essentially from proteid substances, although consisting of oxidation-products of the latter. LANDWEHR and his pupil HIRSCHFELD¹¹ claim that diastase is an animal gum or a similar substance. Doubtless they have had in their hands mainly the polysaccharides which accompany diastase. WRÓBLEWSKI,¹² in a comprehensive research, criticised the earlier investigations, and now believes that he has established beyond doubt that diastase is a *proteid closely allied to the albumoses*. The first preparation which he obtained still consisted of a mixture of proteid with substances of a dextrinous nature, which he succeeded in separating by means of potassium mercuric iodide (*loc. cit.*, 198). The carbohydrate in the mixture proved to be a *pentosan*, which, since it yielded *arabinose* on treatment with dilute sulphuric acid, must be regarded as an *araban*. In a more recent research he has obtained a still purer diastase by fractional "salting out" with ammonium sulphate.¹³

This preparation possessed extraordinary activity, gave most of the proteid reactions (nitric acid, Millon's, biuret, and xanthoproteic reactions), and contained 16.53 per cent. of nitrogen. It was precipitated by *tannin*, as had already been observed

¹ Reychler, *Ber. d. d. chem. Ges.*, xxii., 414, 1889.

² Loew, *Pflüg. A.*, xxvii., 203.

³ Brown and Heron, *Liebig's Ann.*, cxcix., 249.

⁴ Schwarzer, *J. pr. Ch.* [2], i., 212, 1870.

⁵ Osborne, *loc. cit.*

⁶ Seegen and Kratzschmer, *Pflüg. A.*, xiv., 593.

⁷ Bizio, *Comptes Rendus*, lxxv., 175.

⁸ Barth, *Ber. d. d. chem. Ges.*, xi., 474.

⁹ Lintner, *loc. cit.*

¹⁰ Hüfner, *J. pr. Ch.* [2], v., 372.

¹¹ Hirschfeld, *Pflüg. A.*, xxxix., 499.

¹² Wróblewski, *Z. physiol. Ch.*, xxiv., 73.

¹³ Wróblewski, *Ber. d. d. chem. Ges.*, xxxi., 1130, 1898.

by DUBRUNFAUT.¹ The still not completely pure preparation yielded on decomposition with stannous chloride *leucine* and *tyrosine*, and probably also *arginine*.

It is very slightly *diffusible* (KRABBE²), but does not diffuse through the living protoplasm of young germinating seeds of plants (GRÜSS³).

Analyses of diastase have been published by DONATH,⁴ ZULKOWSKI,⁵ and others, but they do not agree, and owing to the undoubted impurity of the preparations are without value.

It is naturally destroyed by acids and alkalies, even in a relatively slight degree of concentration.

It is destroyed by *pepsin*, but not by *trypsin* (WRÓBLEWSKI⁶).

In the dry state it can be heated to about 150° C., but at 158° C. it loses its activity (HUEPPE⁷).

On the other hand, in an aqueous solution it is, like all enzymes, undoubtedly destroyed at about 80° C., but the statements as to the temperatures differ greatly. It is certain that diastase, as is usual with ferments, is protected to a certain extent against the influence of high temperatures by the presence of its specific substratum, so that the decomposition-point is thus raised (LINTNER⁸).

Determination of the Diastatic Power.—Quantitative determinations of the quantities of maltose produced by diastase have been made, *inter alios*, by MUSCULUS,⁹ PAYEN,¹⁰ SCHWARZER,¹¹ O'SULLIVAN,¹² and BARANETZKI.¹³ The last-named was also the first to show that the starch *granules* were also attacked by diastase, the greatest resistance being offered by potato starch (*loc. cit.*, 38).

KÜBEL¹⁴ identified the sugar by the yellow coloration given with sodium hydroxide, and made a comparative estimation of its quantity colorimetrically, with the aid of a solution of potassium chromate.

¹ Dubrunfaut, *Dingler's polyt. Journ.*, cxxxvii., 491, 1868.

² Krabbe, *Jahrb. f. wissenschaft. Bot.*, xxi., 520, 1890.

³ Grüss, *ibid.*, xxvi., 379.

⁴ Donath, *Ber. d. d. chem. Ges.*, viii., 795.

⁵ Zulkowski, *loc. cit.*, 654.

⁶ Wróblewski, *Z. physiol. Chem.*, xxiv., 173.

⁷ Hueppe, quoted by Fermi and Pernossi, *Z. f. Hyg.*, xviii., 83.

⁸ Lintner, *J. pr. Ch.*, N.S., xxxvi., 481, 1887.

⁹ Musculus, *Ann. de Chim. et Phys.* [3], lx., 203.

¹⁰ Payen, *ibid.* [4], iv., 286; vii., 382.

¹¹ Schwarzer, *J. pr. Ch.*, N.S., i., 212, 1870.

¹² O'Sullivan, *Journ. Chem. Soc.*, ii., 125, 1876.

¹³ Baranetzki, *loc. cit.*, 27, *et seq.*

¹⁴ Kübel, *Pflüg. A.*, lxxvi., 276, 1898.

CRIPPS¹ determined the time occupied in the process of the diastatic action of malt extract, which was found to act with different degrees of energy on different kinds of starch. DETMER² tested the progress of the diastatic decomposition by the iodine reaction, which changed from blue, through violet, red, and yellow, to colourless.

WRÓBLEWSKI³ used for this purpose the so-called "soluble starch" which he prepared by his own method. The resulting reducing carbohydrates were heated with Fehling's solution, and the amount of copper which separated estimated by Allihn's method.

A suitable method of estimating the diastatic power of malt extract has also been described by SYKES and MITCHELL.⁴

A method of diastasimetry based upon the disappearance of the iodine reaction has been proposed by ROBERTS.⁵

LINTNER⁶ regards the iodine reactions as unsuitable for comparing the diastatic capacity of different solutions of ferments.

He has therefore worked out a method which takes as its basis the amount of sugar formed from equal quantities of starch.

He allows different quantities of the solution of the ferment under examination to act upon the same quantities of starch, and so determines the quantity of the solution of the ferment which produces so much sugar that a definite amount of Fehling's solution is exactly reduced. As the unit of fermentative power ($F = 100$) he takes a diastase, of which 0.03 c.c. of a solution of 0.1 : 250.0—i.e., 0.12 mgrm.—produces within an hour at the ordinary temperature from 10 c.c. of a 2 per cent. solution of soluble starch, prepared in a definite manner, so much sugar that 5 c.c. of Fehling's solution are exactly reduced. If, for example, he used 0.24 mgrm. of a preparation under the same conditions, this would have a fermentative power $F = 50$, &c.

Conditions of the Action of Diastase.—With the aid of these methods attempts have been made to determine the influence of different agents on the diastatic action. Unfortunately, owing to the different methods of estimation and to other causes, no general agreement has been arrived at on very many questions. Each new investigator in these cases has only helped to swell the immense literature without giving a definite solution of the question, since another has invariably come forward to oppose

¹ Cripps, *Chem. Centralbl.*, i., 324, 1890.

² Detmer, *Z. physiol. Ch.*, vii., 1, 1882.

³ Wróblewski, *Z. physiol. Ch.*, xxiv., 173.

⁴ Sykes and Mitchell, *Analyst*, xxi., 122, 1896.

⁵ Fully described by Gamgee, *Physiol. Ch. d. Verdauung*, loc. cit., 55. See also *Maly's Jb.*, xi., 356.

⁶ Lintner, *J. pr. Ch.*, N.S., xxxiv., 378, 1886.

his statements. Hence we shall only refer to the most important results.

Moreover, the results obtained with *malt diastase* do not agree with those from *saliva diastase*.

The behaviour towards changes of temperature is the normal one. Only one fact is remarkable about it—i.e., that at higher temperatures there is a larger yield of *dextrins*, whilst at lower temperatures (50° to 60° C.) more *sugar* is produced. This fact has been urged in support of the existence of two enzymes.

Whilst the older observers (COHNHEIM,¹ BARANETZKI,² RICHTER³) state that diastase, particularly saliva diastase, is not affected by hydrochloric acid up to a certain concentration, LANGLEY⁴ has shown beyond doubt that it is rendered inactive even by 0.015 per cent. of hydrochloric acid at 40° C. On the other hand, still smaller quantities have a stimulating effect upon its activity (CHITTENDEN and GRISWOLD⁵).

Of other acids carbonic acid has a pronounced stimulating action (MÜLLER-THURGAU⁶) as has also citric acid (DETMER⁷). Salicylic acid is indifferent (KRAUCH⁸), (cf., however, KJELDAHL,⁹ *infra* under *saliva diastase*). Hydrofluoric acid has a markedly injurious influence when in a concentrated solution (EFFRONT¹⁰). According to KJELDAHL⁹ organic acids (lactic, butyric, and acetic acids) in small quantities have a beneficial effect. Boric acid is without influence (LEFFMANN and BEAM¹¹).

Salts of the alkalies and alkaline earths were found by LINTNER¹² to have no influence, contrary to the statements of MAYER and others, who attempted to draw a distinction between the favourable action of *sodium chloride* and the injurious effect of *potassium chloride*. GANS¹³ has observed a retardation in the diastatic decomposition of *glycogen* through the presence of *alkali carbonates*.

The statements as to the influence of the salts of heavy metals are also at variance.

¹ Cohnheim, *Virch. A.*, xxviii., 241.

² Baranetzki, *loc. cit.* in various places.

³ Richet, *Journ. d. l' Anat. et Physiol.*, xiv., 285.

⁴ Langley, *Journ. of Physiol.*, iii., 246; iv., 18.

⁵ Chittenden and Griswold, *Amer. Chem. J.*, iii., 205, 1882.

⁶ Müller-Thurgau, *Thiel's Landw. Jahrb.*, 1885, 795.

⁷ Detmer, *Z. physiol. Ch.*, vii., 1, 1882.

⁸ Krauch, *Landw. Versuchsstat.*, xxiii., 77.

⁹ Kjeldahl, *Z. ges. Brauw.*, 1880, 186.

¹⁰ Effront, *Bull. Soc. Chim.*, [3], iv., 627, 1890.

¹¹ Leffmann and Beam, *Analyst*, xiii., 103, 1888.

¹² Lintner, *J. pr. Ch.*, N.S., xxxvi., 841, 1887.

¹³ Gans, *Verh. Congr. f. inn. Med.*, 1896, 449.

LINTNER (*loc. cit.*) and KJELDAHL (*loc. cit.*) found them to have an extremely injurious influence, particularly the salts of lead, zinc, and iron, whilst EFFRONT¹ found them in some cases to be very advantageous, notably *aluminium acetate* and *vanadium salts*. According to KJELDAHL (*loc. cit.*) borax, alum, and arsenic salts have a prejudicial action, and gypsum very slightly so. The influence of phosphates, proteids, picric acid, and asparagine is also very favourable, according to Effront, though in very different degrees. In like manner he found that an *infusion of ungerminated seeds* had a favourable action, thus lending support to his view that the intensity of the action of diastase is considerably influenced by the presence of foreign substances, and that to this cause alone is to be attributed the varying degrees of activity in the diastases of different origin.

DETMER² has proved that the action of *atropine* is injurious; *strychnine*, according to KJELDAHL (*loc. cit.*), is without influence. Other poisons have been tried by CHITTENDEN³ and his pupils in their comprehensive investigations, and have been found to vary greatly in their action; but I cannot deal more fully with this here.

MORITZ and GLENDINNING⁴ have made a very important observation from a theoretical point of view, and one which establishes for *diastase* a fact in agreement with Tamman's view as to the occurrence of an end-point at which the ferment becomes *inactive* in the case of *emulsin* (see p. 57). They found that *malt diastase* did not decompose the whole of the starch present, but eventually became inactive. On adding *fresh* starch, however, to this mixture the ferment continued its action with *undiminished* intensity, and even did so a third time when certain conditions were observed. These depended upon the quantity of malt extract and, above all, on the *temperature*. At higher temperatures (60° to 65° C.) the decomposing power of the *residual diastase* was considerably less than it was the first time, which was attributed to a gradual decomposition of the ferment. This observation is therefore particularly interesting, because the same result was obtained by the fresh addition of the *same* substratum as Tamman (see p. 57) obtained with a *similar substratum*—i.e., *salicin*—in a mixture of *emulsin* and *amygdalin*.

¹ Effront, *Comptes Rendus*, cxv., 1324, 1892; cxx., 1281, 1895. See also Effront-Bücheler, *Die Diastasen*, 1900, 126.

² Detmer, *Landw. Jahrb.*, x., 757, 1881.

³ Chittenden, *Maly's Jb.*, xv., 256 *et seq.*, 1885.

⁴ Moritz and Glendinning, *Journ. Chem. Soc.*, lxi., 689, 1892.

The closely-connected question whether the activity of the diastase is interfered with by the accumulation of the decomposition-products has a special theoretical interest.¹

Of course, the diastatic action becomes weaker with the advance of the hydrolysis, but in the opinion of many authorities this should *not* be attributed to the accumulation of decomposition-products.

WORTMANN² contends that, as a matter of fact, a suspension of the diastatic activity is physiologically necessary for the plant when a sufficient amount of sugar has been produced, and that we ought therefore, *a priori*, to assume the converse of what has been experimentally established. Otherwise we must fall back upon the view of BARANETZKI,³ who concluded that the production of diastase was intermittent. This view is also in agreement with the statement, which stands in urgent need of corroboration, that the living cell only produces enzymes when it requires their activity, to which we have already referred in the general part. MÜLLER-THURGAU, however, denies that the decomposition-products have no influence.

MÜLLER-THURGAU⁴ has made a fuller investigation of the influence of temperature upon the action of diastase. He finds that at lower temperatures (0° to 20° C.) the amounts decomposed increase in proportion to the time, but that, at higher temperatures, the activity gradually *decreases*, first on account of the increasing *dilution* of the substratum, but also on account of the *disturbing influence* of the accumulated *decomposition-products*. The rate of increase at the higher temperatures is somewhat influenced by this. In comparing the results obtained from an action of short duration only, he found the increase in the activity from 0° C. to 10°, 20°, 30°, and 40° C., to be in the ratio of the following numbers:—

$$7 : 20 : 38 : 60 : 98.$$

Carbonic acid has a pronounced accelerating influence upon diastatic action,⁵ as has also an increase in the *pressure*, but carbonic acid, *accompanied by higher pressure*, was found to be effective in a higher degree than the sum of these factors, inducing the diastase to act energetically upon starch which had not been converted into paste.

¹ Cf. Brown and Heron, *Ann. d. Chem. u. Pharm.*, excix., 247, 1880.

² Wortmann, *Z. physiol. Ch.*, vi., 324.

³ Baranetzki, *loc. cit.*, 62.

Müller-Thurgau, *Thiel's Landw. Jahrbüch.*, 1885, 795.

⁵ According to Detmer, *Z. physiol. Ch.*, vii., 1, 1882.

With regard to the action of sunlight, particularly on *diastase*, a full investigation has been made by GREEN,¹ which is of great interest for the question of the utilisation of the reserve material of plants under the influence of different rays.

He found that the ultra-violet rays were extremely injurious to *diastase*, particularly that of malt; the green rays were also injurious. On the other hand, different parts of the spectrum, notably in the red, orange, and blue, had a stimulating influence, though only temporarily so; afterwards decomposition invariably occurred, the initial acceleration being due to the *zymogen* being converted into the active ferment. The different *diastases* varied in their behaviour, that of malt extract being destroyed to the extent of 68 per cent., saliva *diastase* to 45 per cent., whilst that of extract of leaves was only destroyed to the amount of 8 per cent. Green attributed this to protective influence of the chlorophyll.

Action of Diastase on Different Kinds of Starch.—Whilst *diastase* easily and rapidly liquefies and decomposes gelatinised starch in general, the “raw” starch-granules vary greatly in their resistance to the action of *diastase*. In the cold, barley and wheat starches dissolve fairly rapidly, whilst potato starch hardly dissolves at all. At higher temperatures, too, the different varieties of starch behave in a totally different manner. At 50° C., according to LINTNER,² the following amounts are dissolved:—barley starch, 12 per cent.; maize starch, 2 per cent.; and green malt starch, 29 per cent.; at 55° C., potato starch, 5 per cent.; barley starch, 53 per cent.; green malt starch, 58 per cent.; at 60° C., potato starch, 52 per cent.; barley starch, 92 per cent.; and maize starch, 18 per cent. Frequently these differences are attributed to the physical structure of the starch granules, which, it is argued, also remains to some extent in the paste, and from this the deduction is drawn that the differences in the *dextrins* are also only *physical*; the apparent intermediate stages are hence considered to be so intermingled that the process of saccharification is in different stages in different parts of the starch paste. This view, which is supported by POTTEVIN,³ *inter alios*, would render all the laborious researches on the *dextrins* of no avail, but has hardly sufficient foundation.

The Decomposition of Starch by Diastase.—Notwithstanding the fact that a large number of investigators have attempted to solve this difficult problem, no general agreement on this important question has yet been attained. This is primarily due to the great difficulty of obtaining the products formed in the *diastatic* process in a pure condition and separating them from one another.

¹ Green, *Philosoph. Transact.*, clxxxviii., 167, 1897. ² Lintner, *loc. cit.*

³ Pottevin, *Thèse*, Paris, 1899. *Mon. Scientifique*, 1900, 116.

All that has been definitely established is that in the diastatic decomposition there are formed, on the one hand, reducing sugars, which are *hexabioses*; and, on the other hand, amorphous, non-crystalline *complex polysaccharides* (*dextrins*), which cause little or no reduction, and which when decomposed with acid yield glucose.

As soon, however, as we go beyond these fundamental facts we continually meet with contradictions. Since the workers in the field of research differ from one another in their statements, even on the most essential points, it is not possible to obtain a clear conception of the subject from the results at present at our disposal.

If we consider, in the first place, the nature of the *hexabioses* which are formed, the only point established beyond all doubt is that, in any case, *maltose* is produced. In addition to this, many authorities assert that an isomeric disaccharide, *isomaltose*, is formed, whilst its existence is entirely denied by others. We shall return to this subsequently.

But it is still more difficult to come to a decision on the question of the non-crystallisable parts of the diastatic products, which are designated by the collective name of *dextrins*. Whilst some thrust aside all the differences which have been recorded between the different dextrins as non-essential and recognise only *one* dextrin, others hold the view that there exists a larger or smaller number of dextrins, and almost every one brings forward a view of his own which is not shared by the other authorities. Thus it is almost a hopeless task to define the present position of the question, especially since there is, in addition, considerable confusion in the nomenclature to increase the difficulty.

The name *dextrin* originated with BIOT, who, however, applied it to a substance which still gave a coloration with iodine—i.e., almost the same substance which has subsequently been termed *soluble starch*. BÉCHAMP was the first to give the name of *dextrin* to substances which no longer gave a coloration with iodine.

The first to distinguish *two* dextrins was BRÜCKE.¹ He found that one still gave a red coloration with iodine, and named this *erythro-dextrin*, whilst the other which no longer reacted with iodine he termed *achroo-dextrin*. The existence of the latter, which is identical with NASSE's² *dextrinogen*, has been estab-

¹Brücke, *Sitzb. d. Wiener Acad., Math. Phys. Cl.*, iii., 126, 1872 (gives the older literature).

²Nasse and others, *Pflüg. A.*, xiv., 477, 1877.

lished beyond doubt, whilst the chemical individuality of *erythro-dextrin* is open to question.

To these first accepted dextrins was next added, as the first decomposition product, the so-called *soluble starch*, which still gives a blue coloration with iodine, but is soluble in water.

BONDONNEAU,¹ however, does not regard this as belonging to the dextrins, but as still being starch, though he distinguishes *three* dextrins apart from this. On the other hand, MUSCULUS and GRUBER² asserted that soluble starch was a *decomposition product* belonging to the dextrins.

They also distinguished three achroodextrins (α, β, γ), which, they asserted, differed from one another in their rotation and reducing power. Subsequently the existence of a very large number of dextrins as intermediate steps was assumed, notably by Brown and his collaborators, with which we shall deal more fully in discussing the theories of the decomposition.

On the other hand, BROWN and MILLAR,³ in their latest research, conclude that there is only one well-defined *achroo-dextrin* as a *final product* of diastatic action. Brown had already, at an earlier period,⁴ shown that the diastatic process below 60° C. invariably came to a standstill (or, at any rate, only continued from that point very slowly), at a stage in which the reducing power = 80 per cent., calculated as glucose, and the specific rotation was $[\alpha]_D = 150^\circ$. In this publication Brown and Millar attempt to prove that in this case *only maltose* is produced in addition to a single *achroo-dextrin*, which they have examined more fully.

It is only very slowly further decomposed by diastase, gives a specific rotation of $[\alpha]_D = 197^\circ$ to 198° , and has a *reducing capacity of 5.5 per cent. not to be attributed to the presence of foreign substances*. On cautious oxidation it is converted into an acid, *dextrinic acid*, which points to the presence of a free reducing aldehydic group in this dextrin. They assign to it a constitution, according to which it is produced by the elimination of 39 molecules of water from 40 molecules of glucose.

If these results should be confirmed they will elucidate the whole question of the *achroodextrins*. The question of the nature of *erythro-dextrin* and of the doubtless identical *erythro-granulose* of Wijsman, to which we shall presently return, has been much less thoroughly investigated.

Still more obscure is the so-called *amylodextrin*. It was first

¹ Bondonneau, *Comptes Rendus*, lxxxi., 972, 1210; *Bull. Soc. Chim.*, xxiii., 98, 1875.

² Musculus and Gruber, *Z. physiol. Ch.*, ii., 188, 1878.

³ Brown and Millar, *Journ. Chem. Soc.*, lxxv., 315, 1899.

⁴ Brown and Heron, *Journ. Chem. Soc.*, xxxv., 596, 1879.

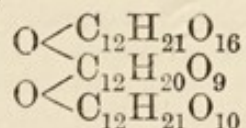
obtained by NÄGELI¹ in the decomposition of starch by very dilute acids in the cold. Afterwards it was frequently regarded as being closely connected with *soluble starch*.

BROWN and MORRIS,² on the other hand, claimed for *amylodextrin* a distinct chemical individuality.

They represented it as a definite substance, to which they assigned the formula $(C_{12}H_{10}O_{10})_6 \cdot C_{12}H_{22}O_{11}$ —a formula which they were also able to confirm by a determination of its molecular weight. It thus contains, in accordance with Brown's hypothesis (*vide infra*), six "amylin" and one "amylon" groups. It is unfermentable, and forms spherical crystals closely resembling *inulin*. They obtained it by a very slow hydrolysis of ungelatinised starch with 0.2 per cent. sulphuric acid; but it is also said to be formed in the diastatic decomposition. Subsequently Brown and Millar³ purified it by converting it into the *nitrate*, and established its identity. It is very closely related to *maltodextrin* (*vide infra*).

A dextrin soluble in dilute alcohol, *maltodextrin*, has been described by several chemists (HERZFELD,⁴ BROWN and MORRIS⁵).

These different *maltodextrins*, however, had few points of agreement. The question of fermentability in particular was answered positively by Herzfeld and negatively by Brown and Morris. Then, afterwards, the existence of such a *maltodextrin* was again absolutely denied by SCHIFFERER,⁶ who regarded the substance obtained as an impure *isomaltose*. BROWN⁷ and his co-workers, however, strongly adhere to its existence, and, in turn, assert that *isomaltose* is only a mixture of maltodextrin and maltose. They assign to *maltodextrin* the formula



with one free COH group. The question is still far from being settled.

BEYERINCK⁸ has increased the nomenclature with a new dextrin, *leucodextrin*, which is somewhat more resistant to the action of diastase, and

¹ Nägeli, *Beiträge z. Kenntniss der Stärkegruppe*, Leipzig, 1874, quoted by Brown and Morris. See next reference.

² Brown and Morris, *J. Chem. Soc.*, lv., 449, 1889; and *Z. f. d. ges. Brauw.*, 1889, 437.

³ Brown and Millar, *J. Chem. Soc.*, lxxv., 311, 1899.

⁴ Herzfeld, *Ueber Maltodextrin*, Inaug. Diss. Halle, 1879.

⁵ Brown and Morris, *Journ. Chem. Soc.*, xlvii., 527, 1885 (gives the older literature).

⁶ Schifferer, *Die nichtkrystallisirb. Prod. d. Einw. von Diastase auf Stärke*, Diss. Basle, 1892.

⁷ Brown and Millar, *Journ. Chem. Soc.*, lxxv., 1899, 286, 315.

⁸ Beyerinck, *C. f. Bakt. (II.)*, i., 221, 1895.

precipitated by even fairly dilute alcohol. He himself states that it is not a chemical individual, and intends the collective name only to indicate a genetic relation to Nägeli's starch cellulose.

We have thus to enumerate the following dextrins, not including *soluble starch*.

1. *Erythrodextrin* (including Wijsman's erythrogranulose) is decomposed further by diastase, and is precipitated by alcohol even in slight concentration; it gives a reddish-violet coloration with iodine.

2. *Achroodextrins* are not further decomposed by diastase, although probably so by *maltase*; they can be precipitated by alcohol, give no coloration with iodine, and have slight reducing power.

3. *Amylodextrin* of Brown and Morris is decomposed further by diastase, causes reduction, and is only precipitated by more concentrated alcohol. It gives a purple coloration with iodine, has an optical rotation four times as great as that of glucose, and can be isolated from its aqueous solution by freezing.

4. *Maltodextrin* of Brown and Morris is soluble in alcohol. Its existence is doubtful.

All dextrins are characterised by not being easily crystallised, by their ready solubility in water, and by rotating the beam of polarised light to the right. Acids convert them into glucose.

In direct opposition to these views we have the conclusions of POTTEVIN,¹ who attributes the observed differences in the various dextrins to the presence of different quantities of *maltose*, as also to the want of homogeneity in the original starch granules. In particular, he denies the existence of a maltodextrin as a chemical substance, since he claims to have decomposed it by fractional precipitation with alcohol into another dextrin and maltose. By fractional precipitation with alcohol he obtained an indefinite number of dextrins, which passed through all the stages from soluble starch to maltodextrin, and, on further decomposition, yielded considerable quantities of maltose. The iodine coloration was found to depend largely upon the size of the original starch granules.

Glycogen appears to behave somewhat differently. Although, when decomposed by acids, it yields products which correspond to *soluble starch* and *erythrodextrin*, in the diastatic decomposition there is only one product resembling *achroodextrin*, which is not further decomposed by diastase, *dystropodextrin*, but no substance corresponding to *erythrodextrin*. *Liver diastase*, however, also forms some *erythrodextrin* (TEBB²).

¹ Pottevin, *Thèse*, Paris, 1899. Quoted in *Moniteur Scientif.*, 1900, 116. Cf. *Comptes Rendus*, cxxvi., 1219, 1898.

² Tebb, *Journ. of Physiol.*, xxii., 427, 1898.

Maltose.—The reducing sugar which results from the action of diastase was at first regarded as *glucose*:¹ DUBRUNFAUT² was the first to discover that the sugar produced in the process differed from glucose, and named it *maltose*. This important observation was completely forgotten, and was first brought into notice again by O'SULLIVAN.³ Through the researches of MAERCKER,⁴ E. SCHULZE,⁵ and others (cf. *saliva diastase*), it was definitely established that a sugar differing from glucose was formed here, and this has since been generally termed *maltose*.

According to the nomenclature of E. Fischer, *maltose* is a *glucobiose* of the formula $C_{12}H_{22}O_{11}$, which reduces Fehling's solution and has a much greater specific rotation than glucose.

It is not attacked by diastase, but is decomposed by an enzyme adapted to it (*maltase*, *q.v.*) into two molecules of *d-glucose*.

It does not reduce *neutral copper acetate* (Barfoed's reagent).⁶

Iso-Maltose.—In addition to maltose, an isomeric glucobiose, iso-maltose, is said to be formed by the action of diastase; it shows other characteristics, and is fermented with more difficulty by yeast. It was first prepared synthetically from glucose by E. FISCHER.⁷ Then it was discovered by LINTNER⁸ as a product of the diastatic decomposition of starch, and was more fully examined.

Although BROWN and MORRIS⁹ deny its separate existence, and assert that it is an impure maltose, the other authorities adhere firmly to its individuality.¹⁰ It is not yet known, however, in a pure condition.

Theories of the Decomposition.—As regards the chemical process of the decomposition of starch, there has been much discussion. No absolute proof of any of these theories can be given so long as the constitution and the size of the molecules of starch and the dextrins are unknown.

¹ It was first discovered by Guerin-Varry, *Ann. Chim. Phys.* (2), xxxvi., 225.

² Dubrunfaut, *Ann. d. Chim. et Phys.* [3], xxi., 178, 1847.

³ O'Sullivan, *Journ. Chem. Soc.*, 1876, ii., 125.

⁴ Maercker, *Thiel's Landwirthsch. Jb.*, 1877. Suppl. part., 286.

⁵ E. Schulze, *Ber. d. d. chem. Ges.*, vii., 1047.

⁶ Musculus and v. Mering, *Z. physiol. Ch.*, ii., 403, 1878.

⁷ E. Fischer, *Ber. d. d. chem. Ges.*, xxiii., 3687, 1890; cf. Scheibler and Mittelmeier, *Ber. d. d. chem. Ges.*, xxiv., 303, 1891.

⁸ Lintner, *Z. f. ges. Brauwesen*, 281, 1891; 6, 123, 1892; Lintner and Düll, *Z. f. angew. Ch.*, 263, 1892; Lintner, *Z. f. d. ges. Brauw.*, 414, 1894; cf. Ling and Baker, *Journ. Chem. Soc.*, lxxvii., 702, 739.

⁹ Brown and Morris, *Journ. Chem. Soc.*, lxxvii., 709, 1895.

¹⁰ *Vide, inter alios*, E. Fischer, *Z. physiol. Ch.*, xxvi., 71, 1898.

At first it was believed that there was a successive transformation of starch into *dextrin*, and of dextrin into *sugar*.

Musculus, in the publications referred to above, stubbornly upheld the theory of the decomposition into dextrin and maltose against numerous opponents until he finally succeeded in conjunction with Gruber in giving absolute proof that the *starch* was decomposed into dextrin and maltose.

BROWN and HERON,¹ in their comprehensive research, ascribed to "soluble starch" the formula $(C_{12}H_{20}O_{10})_{10}$. By the addition of water this is transformed into *erythrodextrin* $(C_{12}H_{20}O_{10})_9$ and one molecule of $C_{12}H_{22}O_{11}$ (*maltose*). Thus there is a gradual splitting-off of maltose until finally on the eighth hydrolysis the process comes to a stand-still. At that stage 81 per cent. of maltose and 19 per cent. of the last achroodextrin are present. BOURQUELOT'S² view was, in the main, in accordance with theirs.

BROWN and MORRIS³ subsequently brought forward a complicated theory. In their conception the four dextrin groups are arranged about a fifth, and in the decomposition the whole structure falls to pieces. These five dextrin groups have each the constitution $(C_{12}H_{20}O_{10})_{20}$, and this dextrin which gives no coloration with iodine, and thus corresponds to achroodextrin, is the only true dextrin. Moreover, they also assume the existence of numerous intermediate products, the *amyloïns*, which consist of a combination of *amylin* groups, $C_{12}H_{20}O_{10}$, and *amylon* groups, $C_{12}H_{22}O_{11}$. For instance, *amylodextrin*, according to this theory, consists of 6 amylin and 1 amylon; *maltodextrin* of 2 amylin and 1 amylon. There are also in their opinion many other amyloïns which, *inter alia*, take part in the secondary fermentation of beers. LINTNER and DÜLL⁴ have subjected this theory to a vigorous criticism, their main conclusion being that the *amyloïns* are varying mixtures of *dextrins* and *iso-maltose* (*vide supra*). They consider that in the diastatic decomposition there are formed *three dextrins* which correspond to the old conception of *amylodextrin*, *erythrodextrin*, and *achroodextrin*, and two glucobioses, *maltose* and *iso-maltose*, which latter they claim to have detected in Brown's maltodextrin.

Theory of two Enzymes.—An essentially different theory of the action of malt extract on starch is that proposed by WIJSMAN.⁵ He considers that there is not one simple ferment, *diastase*, but assumes the existence of two separate enzymes, to which he

¹ Brown and Heron, *Liebig's Ann.*, cxcix., 165, 1880.

² Bourquelot, *Comptes Rendus.*, civ., 576.

³ Brown and Morris, *Journ. Chem. Soc.*, lv., 462, 1889; lxix., 709, 1895.

⁴ Lintner and Düll and others, *Ber. d. d. chem. Ges.*, xxvi., 2533, 1899; Schifferer, *Diss.*, Basle, 1892.

⁵ Wijzman, *Recueil d. Trav. Chim. des Pays-Bas*, ix., 1.

assigns the names of *maltase* and *dextrinase*. According to him, this division of diastatic ferments had previously been definitely postulated by CUISINIER, who had also chosen the names applied to the separate enzymes. Both names are now obviously untenable. *Maltase* is the name rightly applied to the enzyme which decomposes maltose; and *dextrinase*, according to the principles of ferment nomenclature, should denote a ferment which *decomposes* dextrins. On the contrary, Wijsman assumes that the dextrinase converts into *malto-dextrin* in a secondary reaction the *erythrogranulose* which is first formed from starch, together with *maltose* by the action of his *maltase*. *Erythrogranulose* is thus, in addition to *maltose*, a primary product of the action of the ferment.

Wijsman demonstrates the action of his two ferments by means of his diffusion method previously mentioned. On applying a few drops of a solution of diastase to a gelatin plate impregnated with starch they spread concentrically. If, now, iodine be added, there is left in the centre, where both the ferments have acted, an uncoloured spot, which is surrounded by a violet-ring; the latter, he asserts, represents the exclusive zone of action of the "*maltase*" (production of *erythrogranulose*), whilst the colourless central zone represents the sphere of the activity of the "*dextrinase*." Where the ferments have not acted at all the blue colour of the starch iodide is naturally pronounced.

He further states that *maltase* becomes inactive as low as 55°C., whilst *dextrinase* resists temperatures up to 75° C. This is opposed to numerous statements that on heating diastatically active solutions the capacity of producing more deeply-seated changes is weakened to a greater extent than the power of forming the first decomposition products.

Dextrinase is stated to occur exclusively in the exterior zone of germinated barley corn; he therefore claims to have isolated *pure* malt "*maltase*" without dextrinase, and by its means to have prepared from starch *pure erythrogranulose* in addition to maltose. *Erythrogranulose* is readily soluble in water, but is completely different from *amylodextrin*.

BEYERINCK¹ has accepted this view in the main, but concludes that *dextrinase* does not exist ready formed in malt extract, but is formed on heating from yet another enzyme, "*granulase*," which produces maltose and achroodextrin (maltodextrin); on heating, the "*granulase*"—i.e., the dextrin-forming function—remains intact, whilst the maltose-producing function is destroyed. Other granulases are not so sensitive, from which the deduction can be drawn that there is a difference in the granulases of different origin.

¹ Beyerinck, *C. f. Bakt.* (II.), i., 229, 1895.

He distinguishes between "*alkali granulases*," to which belong *saliva diastase* and *pancreas diastase*, and "*acid granulases*." To the latter belong the granulases of wheat, rye, and barley, and differing from these the *granulase of buck wheat*, which may be regarded as the prototype of the dicotyledon granulases, *maize granulase*, and that of the "*granulobacter*."

His "maltase" occurs widely distributed throughout the vegetable kingdom. It decomposes starch into *erythrodextrin* and *maltose*. It would be decidedly more practical to designate these two enzymes as *erythroamylase* (granulase) and *achrooamylase* (maltase), if they actually exist.

POTTEVIN¹ also believes that the decomposition takes place in two successive stages. He, too, concludes that there are *two distinct enzymes*, a *dextrinase* which transforms the starch into dextrin, and the true *amylase* which completes the further transformation into maltose.² This notion appears to me very unhappy. Apart from the fact that both enzymes would have to occur quite constantly everywhere, which, indeed, is conceivable, it is still not clear why the *amylase invariably* transforms a considerable portion of the dextrin, and yet always leaves a *definite* residue unaffected. Why does it *invariably* cease to exercise its activity on attaining the same ratio of dextrin to maltose? In support of his view he brings forward the fact that when diastase is heated for a short time (five minutes) at 80° C. it loses its sugar-producing function, while its power of dissolving starch is not affected. We have here rather to deal with a premature production of the final condition in the sense of Tamman, due to a weakening of the ferment. The question of the existence and nature of two enzymes in diastatically-active solutions requires further elucidation.

Occurrence of Diastatic Ferments in the Vegetable World.—The diastatic ferments are widely distributed throughout the vegetable kingdom.

Apart from germinating barley, in which, practically, they play their most important part, they are also found in other germinating seeds, such as oats, wheat, maize, rice, potato tubers

¹ Pottevin, *Thèse*, Paris, 1899, quoted in *Moniteur Scientif.*, 116, 1900.

² The names are quite unpractical. As regards *dextrinase*, which, moreover, is none other than that of Wijsman, the existence of which is denied by Beyerinck, *vide supra*. But how a *dextrin-decomposing, maltose-producing* enzyme comes by the name of *amylase* is absolutely unintelligible, since it has nothing whatever to do with starch. It would be fitting here to use the name *dextrinase*, and to designate as *amylase* the *only starch-decomposing* ferment—*i.e.*, assuming that Pottevin's conclusions are correct.

(PAYEN and PERSOZ¹), vetches, &c. (v. GORUP-BESANEZ²); also in *ungerminated* seeds, though much more sparingly (KJELDAHL³) in fir pollen (ERLENMEYER⁴), the buds of *Ailanthus glandulosa* (PAYEN and PERSOZ¹), &c.

HANSEN⁵ discovered them in the sap of *Ficus carica*. BARANETZKI,⁶ in particular, succeeded in finding them in the organs and juices of very many plants; he found them widely-distributed in seeds, tubers, leaves, roots, &c.; they occur, as a rule, in saps which contain starch (MULDER⁷).

They were found in leaves by BRASSE⁸ and in beetroot (*Beta vulgaris*) by GONNERMANN.⁹

Thus there was soon a tendency to adopt the conclusion that diastase was invariably present in plants where starch was decomposed, and to assign to it the important function of being the instrument of the entire starch-dissolving activity of plants—a view which was put forward, notably, by DETMER.¹⁰

Diastase, however, is probably not to be found *universally* in the parts of plants which contain starch; KRAUCH,¹¹ for example, could not discover it at all in onions and in the vegetative organs of the *birch tree*, and, in the case of the horse-chestnut, found it *only* in the wood. WORTMANN,¹² in particular, energetically opposed the view that the entire decomposition of starch in plants was to be attributed to the activity of unorganised ferments. He discounted the significance of their presence by the fact that, in the first place, he detected them in organs which produced no starch, and where they must therefore, in his opinion, have had no functions; whilst, on the other hand, he laid stress upon the fact that he had failed to discover them in places where particularly active transformations of starch took place, as, for instance, in *green leaves*, in which, unlike Baranetzki and Brasse, he could find little or no diastase. This part of his

¹ Payen and Persoz, *Ann. d. Chim. et Phys.*, liii., 73; lvi., 537; lx., 441.

² v. Gorup-Besanez, *Ber. d. d. chem. Ges.*, vii., 1478; viii., 1510.

³ Kjeldahl, *Resum. d. Labor. Carlsberg*, quoted by Brown and Morris, *Journ. Chem. Soc.*, lvii., 505.

⁴ Erlenmeyer, *Münch. Acad. Sitzb.*, ii., 204, 1874.

⁵ Hansen, *Arb. Bot. Inst. Würzb.*, iii., 271.

⁶ Baranetzki, *Die Stärkeumbild. Fermente*, Leipzig, 1874.

⁷ Mulder, *Chem. des Bières*, translated by Grimm, Leipzig, 226, 1858.

⁸ Brasse, *Comptes Rendus*, xcix., 878.

⁹ Gonnermann, *Chemiker Ztg.*, 1895, 1806.

¹⁰ Detmer, *Landwirthsch. Jahrbücher*, x., 757, 1881.

¹¹ Krauch, *Landwirthsch. Versuchsstat.*, xxiii., 77.

¹² Wortmann, *Botan. Zeitg.*, xlviii., No. 37, *et seq.*

proof, however, was refuted by BROWN and MORRIS,¹ who, in a very thorough investigation, demonstrated the occurrence of diastatic ferments in green leaves.

Of course, this does not prove that it is really only the *enzyme* diastase which invariably causes the starch to dissolve. We cannot by this disprove experimentally Wortmann's view that a very considerable part of the solution of the starch is effected directly by the living plant cell. Wortmann's assumption is that, in general, the cell *itself* fulfils this function, and that the enzymes are only excreted with the object of dissolving starch which is not directly accessible to the living cell; he also regards this enzyme as being not *completely devoid of life*, but believes that it is still endowed with a "residue" of vital energy derived from the living protoplasm. We have already considered this view in the general part, and have pointed out that it is much too obscure to serve as a *heuristic* principle for the explanation of the action of enzymes.

According to BROWN and MORRIS,² we have to recognise in plants three different diastases, which differ in their action and physiological significance—viz., *secretion diastase*, *translocation diastase*, and *cytase*. GRÜSS³ partially confirms these assertions, but differs from them in other points. One of these three diastases is stated to also have a solvent action on the cell walls (cf. *cytase*). *Secretion diastase* is that of germinating seeds, whilst *translocation diastase* is that of ungerminated seeds and other organs of the plant. They are distinguished by the fact that translocation diastase is not capable of dissolving ungelatinised starch granules, as is done by secretion diastase.

The Secretion of Diastase in Plants.—The proof of the conception that diastase is not produced diffusedly in the organs of the plant, but is an actual *secretion-product*, has been furnished by HABERLANDT.⁴ In confirmation of the assertions of TANGL⁵ and others, he showed that the glutinous envelope of the endosperm of gramineæ invariably excretes diastase. AIMÉ-GIRARD⁶ had similarly shown that diastase is only produced in the exterior layers of the germ and not in the interior layers where the starch is deposited. The ferment and substratum are thus *separate*—a fact which we shall also observe again in the case of

¹ Brown and Morris, *Journ. Chem. Soc.*, lxiii., 604, 1893; gives the literature on the subject of diastase in leaves.

² Brown and Morris, *Journ. Chem. Soc.*, lvii., 507, 1890.

³ Grüss, *Jahrb. wissenschaft. Bot.*, xxvi., 379.

⁴ Haberlandt, *Ber. d. d. botan. Ges.*, viii., 40.

⁵ Tangl, *Sitzb. Wien., Math. Naturw. Cl.*, xcii., 72.

⁶ Aimé-Girard, *Ann. d. Phys. et Chim.* (6), iii., 289, 1884.

emulsin, &c. Haberlandt has discovered them in the cells of the endosperm, and considers it certain that these cells do not belong to the *storage system* of the plant, but represent a true *glandular tissue*, the specific function of which is to *produce* and to *excrete diastase*, like the salivary glands of animals, and which has its analogy, as regards the rest of the vegetable kingdom, in the *pepsin glands* of *insectivorous plants*, the *emulsin-producing groups of cells* of the leaves of *Laurocerasus*, &c.

BROWN and MORRIS¹ were unable to altogether confirm his conclusions; they showed that, in germinated barley, the diastase was secreted by the absorptive epithelium of the scutellum, and that an embryo which had been deprived of this epithelium no longer possessed the power, after the removal, of attacking starch, like that of the uninjured embryo.

As regards the changes perceptible under the microscope, which the starch within the plant undergoes under the influence of the diastase, full investigations have been made, notably by KRABBE,² A. MAYER,³ and GRÜSS,⁴ but I cannot do more than allude to them here.

As a rule, diastase appears to occur in the higher plants as a *zymogen*, for very frequently fresh aqueous extracts are inactive, but, on standing or acidification, become more or less active (BARANETZKI,⁵ BROWN and MORRIS,⁶ REYCHLER⁷). FRANKHAUSE⁸ found that formic acid was produced in the germination of barley, and this has doubtless some significance for the action of the enzyme.

Diastase in Cryptogams.—Saccharifying ferments were first discovered in cryptogams (algæ, mould-fungi, &c.) by KOSMANN.⁹

DUCLAUX¹⁰ discovered diastase in moulds (*Aspergillus niger*); WORTMANN¹¹ in the *tan-mould* (*Fuligo septica*).

The moulds which cause wood to decay (e.g., *Trametes radiciperda*, Hartig) also appear to form diastatic ferments.¹²

¹ Brown and Morris, *Journ. Chem. Soc.*, lvii., 493, 1890.

² Krabbe, *Jahrb. f. wissenschaft. Bot.*, xxi.

³ A. Mayer, *Unters. üb. d. Stärke Körner*. Quoted by Grüss.

⁴ Grüss, *loc. cit.*; and *Festschrift f. Schwendener*, Berlin, 184, 1899.

⁵ Baranetzki, *loc. cit.*

⁶ Brown and Morris, *loc. cit.*

⁷ Reychler, *Ber. d. d. chem. Ges.*, xxii., 414, 1889.

⁸ Frankhause, *Der Bund. Berne*, xxxvii. Quoted by Green, *Ann. of Bot.*, vii.

⁹ Kosmann, *Bull. Soc. Chim.*, xxvii., 251, 1877.

¹⁰ Duclaux, *Chim. Biolog.*, 142 and 164. Quoted by Bourquelot, *Bull. Soc. Mycol. de France*, 230, Supplement.

¹¹ Wortmann, *Z. physiol. Ch.*, vi., 324.

¹² Cf. Hartig, *Die Zersetzungserschein. d. Holzes*, Berlin, 23, 1878.

At a later period the mould-fungi were systematically examined for ferments, notably by BOURQUELOT,¹ in numerous researches, and *diastase* found to be also widely distributed in them. The occurrence of diastatic ferments in *yeast infusions* is technically of special importance. Yeast contains all the ferments which transform starch into glucose, so that indirectly it is capable of fermenting starch, even though it only contains a slight quantity of diastase.

Various species of *Mucor* also contain diastase, so that they can directly ferment starch and dextrins (GAYON and DUBOURG²), and the same remark applies to *Actinomyces* (FERMI³).

A peculiar symbiosis of diastase-producing mould-fungi with alcohol-producing saccharomycetes is found in the yeasts which are used by the inhabitants of Eastern Asia in the manufacture of alcoholic beverages, especially from rice.

The best known is the *koji yeast*, which yields *saki*, the Japanese rice wine. It contains a mould-fungus, *Aspergillus oryzae*, which produces a diastatic ferment, *taka diastase*, although, according to later researches, it is not to be regarded as the cause of the production of alcohol (see *Alcoholic Fermentation*). Taka diastase was first described by ATKINSON,⁴ and was afterwards more closely examined by KELLNER, MORI, and NAGAOKA,⁵ and by BÜSGEN.⁶ WRÓBLEWSKI⁷ has attempted to prepare it in a pure condition, but no continuation of his preliminary communication has as yet appeared. According to the investigations of STONE and WRIGHT,⁸ as also of TAKAMINE,⁹ it acts much more energetically than malt diastase upon starch. It is also not so sensitive to the action of hydrochloric acid as malt diastase and saliva diastase, and is therefore prescribed in certain diseases as a remedy for defective secretion of saliva (LEO¹⁰). Lactic acid, on the other hand, has a pronounced restrictive action.

We find very similar properties in the case of *Tonkin yeast*, which has been examined by CALMETTE¹¹ and SANGUINETTI.¹²

¹ Bourquelot, *Bull. Soc. Mycol. d. France*, ix., 230 ; x., 235.

² Gayon and Dubourg, *Ann. Past.*, i., 532, 1886.

³ Fermi, *C. f. Bakt.*, xii., 713.

⁴ Atkinson, *Monit. Scientif.*, 7, 1882.

⁵ Kellner, Mori, and Nagaoka, *Z. physiol. Ch.*, xiv., 297.

⁶ Büsgen, *Ber. d. d. bot. Ges.*, iii., 66, 1885.

⁷ Wróblewski, *Ber. d. d. chem. Ges.*, xxxi., 1132, 1898.

⁸ Stone and Wright, *Journ. Amer. Chem. Soc.*, xx., 637 ; *Maly's Jb.* 720, 1899.

⁹ Takamine, *Maly's Jb.*, 721, 1899.

¹⁰ Leo, *Therapeut. Monatsh.*, x., 635.

¹¹ Calmette, *Ann. Inst. Past.*, vi., 604, 1892.

¹² Sanguinetti, *Ann. Inst. Past.*, xi., 264, 1897.

There is present in this a mould-fungus which is at least closely allied to the mucors, *Amylomyces Rouxii*, which produces diastase in abundance, together with certain *Saccharomycetes* resembling *S. pastorianus*, which induce alcoholic fermentation. According to Sanguinetti, amylomyces diastase is weaker than taka diastase.

Successful attempts have also been made to *isolate* diastatic ferments from bacteria—e.g., from cholera vibrios, BITTER,¹ WOOD,² and from *B. mesentericus vulgatus* (VIGNAL³). WORTMANN⁴ obtained it to a notable extent from *Bacterium termo* in the form of a substance soluble in water, and precipitable by alcohol. The "bacille amylozyme" of PERDRIX⁵ also produces diastase.

In fact, nearly all micro-organisms possess the power of dissolving starch,⁶ and FERMI⁷ has proved definitely that we have here not *vital* but *enzymic* processes.

¹ Bitter, *Arch. f. Hygiene.*, v.

² Wood, *Labor. Rep. Royal Coll. Phys.*, Edinburgh (II.), quoted by Green, *Annals of Bot.*, vii., 120.

³ Vignal, *ibid.*

⁴ Wortmann, *Z. physiol. Ch.*, vi., 287, 1882.

⁵ Perdrix, *Ann. Inst. Past.*, 287, 1891.

⁶ Cf., Pick, *Wiener klin. Woch.*, 89, 1889.

⁷ Fermi, *Arch. f. Hygiene*, x., 1; *C. f. Bakt.*, xii., 713.

CHAPTER XV.

DIASTATIC FERMENTS IN THE BODIES OF ANIMALS.

AN important part is also played by diastatic ferments in the digestive process of animals, since they transform the insoluble non-absorptionable starch and reserve glycogen into soluble available sugars. They are found, in particular, in the saliva, intestinal secretion, liver, and pancreas.

They are true *enzymes*. GOLDSCHMIDT,¹ alone, expresses the opinion that the diastatic power of the saliva may also be due to *organised ferments*, and has discovered a *mould-fungus* derived from the air, the diastatic activity of which, he asserts, assists that of the saliva. The *bacteria of the mouth* themselves do *not*, as a rule, have a saccharifying action (MILLER²).

The Diastatic Ferment of the Saliva.—The organic part of the saliva was designated *ptyalin* by BERZELIUS³; afterwards this name was transferred to the *diastatic ferment* of the saliva.

The power which the saliva possesses of dissolving starch and converting it into reducing sugars was discovered by LEUCHS,⁴ and soon afterwards was thoroughly investigated by SCHWANN.⁵ MIALHE⁶ precipitated the active principle with alcohol. COHNHEIM⁷ removed it from its solution by means of calcium phosphate, as in Brücke's method, dissolved it in water, and precipitated it with alcohol.

Saliva diastase yields practically the same decomposition-products as vegetable diastase. Here, too, *glucose* was at first believed to be formed until it was recognised through the researches of SEEGEN⁷ and NASSE⁸ that the sugar was distinct from glucose. Nasse named it *ptyalose*, and regarded it as

¹ Goldschmidt, *Z. physiol. Ch.*, x., 294, 1886.

² Quoted by Schlesinger, *Virch. A.*, cxxv., 146, where is given a comprehensive bibliography of the literature on saliva.

³ Leuchs, *Kastner's Archiv f. ges. Naturlehre*, 1881, quoted by Schlesinger, *loc. cit.*

⁴ Schwann, *Poggendorff's Ann.*, xxxviii., 359.

⁵ Mialhe, *Comptes Rendus*, xx., 954.

⁶ Cohnheim, *Virch. A.*, xxviii., 241, 1865.

⁷ Seegen, *Centralbl. med. Wiss.*, 949, 1876.

⁸ Nasse, *Pflüg. A.*, xiv., 477.

different from maltose. v. MERING and MUSCULUS,¹ however, subsequently prepared maltose from the products of the reaction, this sugar being formed *at first exclusively* in addition to dextrin,² as was also found by KÜLZ³ to be the case in the action of saliva on glycogen. They also found *isomaltose*.⁴

Of course, *glucose* is also formed from the *maltose* by the further action of the saliva, since the latter also contains *maltase* (q.v.) It does not, however, contain *invertase*.

Saliva diastase does not appear to be absolutely identical with malt diastase, a fact to which DUFRESNE⁵ was the first to call attention.

The difference is said to be, on the one hand, in its *thermal "death point,"* for it becomes inactive at 65° to 70° C., or, according to BOURQUELOT,⁶ even as low as 58° C., and is thus considerably more sensitive than malt diastase; this point, however, according to BIERNACKI,⁷ greatly depends upon its degree of concentration. Then, it is stated to be unaffected by salicylic acid in a concentration of less than 1 per cent., whilst *malt diastase* becomes inactive even in a 0.05 per cent. solution of the acid (MÜLLER⁸).

Lastly, neutral salts are said to have a different action upon it (NASSE⁹).

On the other hand, PUGLIESE¹⁰ altogether denies that there is any difference between the two diastases. Their properties, he contends, are so much influenced by impurities that no great importance should be assigned to observed differences.

The activity of the diastase is *increased* by *dilute acid*¹¹ (SCHIERBECK¹²), but is rendered inactive by hydrochloric acid of the concentration of that of the gastric juice (KÜBEL¹³).

This opens up the important question whether the diastatic action of the saliva is limited to the cavity of the mouth, or still continues in the stomach. (For the older literature, see SCHLESINGER¹¹.) It appears as though it remains intact for a certain time (KÜBEL¹³); according to RICHET,¹⁴ it acts even more

¹ Musculus and v. Mering, *Z. physiol. Ch.*, ii., 403.

² v. Mering, *Z. physiol. Ch.*, v., 185, 1881. ³ Külz, *Pflüg. A.*, xxiv., 81.

⁴ See also Hamburger, *Pflüg. A.*, lx., 545.

⁵ Dufresne, *Comptes Rendus*, lxxxix., 1070.

⁶ Bourquelot, *Comptes Rendus*, civ., 71. ⁷ Biernacki, *Z. f. Biol.*, xxviii., 49.

⁸ Müller, *J. pract. Ch.*, N.S., x., 444. ⁹ Nasse, *Pflüg. A.*, xi., 156, 1875.

¹⁰ Pugliese, *Pflüg. A.*, lxix., 115 (gives the literature).

¹¹ Cf., however, on this point Schlesinger, *Virch. A.*, cxxv., 167.

¹² Schierbeck, *Scand. Arch. f. Phys.*, iii., 344; cf., Chittenden and Griswold, *Amer. Chem. Journ.*, iii., 205, 1882.

¹³ Kübel, *Pflüg. A.*, lxxvi., 276.

¹⁴ Richet, *Journ. de l'Anat. et Phys.*, xiv., 285.

energetically in the stomach than in the mouth, *though eventually it is undoubtedly destroyed*. (LANGLEY¹).

The introduction of carbon dioxide into a neutral solution has also an injurious influence (EBSTEIN and SCHULZE²), though this is opposed to the statements of DETMER³ and NASSE,⁴ who found that carbon dioxide had an accelerating effect, whilst other gases were indifferent.

Solutions of alkalies, including *sodium carbonate* (CHITTENDEN and ELY⁵) have a restrictive influence (KÜBEL⁶); when carbon dioxide is passed into alkaline solutions, this injurious influence is partially removed. The diastase is thus not destroyed by the alkali, but only enters into combination with it, and is rendered inactive (EBSTEIN and SCHULZE⁷).

Neutral salts, for the most part, have a stimulating influence, notably common salt up to a certain degree of concentration, which depends upon the concentration of the starch paste (KÜBEL⁶).

Magnesium sulphate and mercuric chloride have a pronounced injurious action, whilst *tartar emetic* is beneficial in the same degree of concentration (CHITTENDEN and PAINTER⁸). *Arsenious acid* has no action (SCHÄFFER and BÖHM⁹); *peptones* have a beneficial influence (CHITTENDEN and ELY⁵); *alkaloids* act both ways (NASSE¹⁰); and *antipyrin* is without influence. *Paraldehyde* has a considerable restrictive action, and *thalline sulphate* in very slight quantities has a stimulating effect, but is injurious even in a 1 per cent. solution (CHITTENDEN and STEWART¹¹).

Alcohol checks the fermentation (WATSON¹²), as is also the case with *thymol* (SCHLESINGER¹³), *salicylic acid* exceeding 1 per cent. (MÜLLER¹⁴), and *chloroform*, but not *toluene* (PUGLIESE¹⁵).

The optimum temperature is about 45° C. As regards the

¹ Langley, *Journ. of Phys.*, iii., 246.

² Ebstein and Schulze, *Virch. A.*, cxxxiv., 475.

³ Detmer, *Z. physiol. Ch.*, vii., 1, 1882.

⁴ Nasse, *Pflüg. A.*, xv., 477, 1887.

⁵ Chittenden and Ely, *Journ. of Phys.*, iii., 327.

⁶ Kübel, *loc. cit.* (gives the literature).

⁷ Ebstein and Schulze, *loc. cit.*

⁸ Quoted by Schlesinger, *Virch. A.*, cxxv., 170.

⁹ Schäffer and Böhm, *Wörzb. phys. med. Ges.*, N.S., iii., 238.

¹⁰ Nasse, *Pflüg. A.*, xi., 145, 1875.

¹¹ Chittenden and Stewart, *Maly's Jb.*, xx., 248, 1890.

¹² Watson, *Journ. Chem. Soc.*, xxxv., 539, 1879.

¹³ Schlesinger, *Virch. A.*, cxxv., 340.

¹⁴ Müller, *J. Prakt. Chem.*, N.S., x., 444.

¹⁵ Pugliese, *Pflüg. A.*, lxix., 115.

thermal death-point, *vide supra*. A temperature of -20° C. does not injure the enzyme (PASCHUTIN¹).

The Mode of Action of Saliva Diastase.—The products of the diastatic action of saliva upon starch are:—*Maltose* and *Dextrins* (for further particulars see the preceding description of *Diastase* in general).

HAMMARSTÉN² found that while starch paste of the most different varieties of starch was acted upon by saliva in the same time, the ungelatinised starch granules varied very greatly in the extent to which they were dissolved, *potato starch* being attacked far *more slowly* than *maize starch*; the other starches gave intermediate values. *Finely-pulverised* potato-starch, however, was rapidly dissolved, as was also *masticated* starch. *Uninjured* starch granules are not attacked at all according to BOURQUELOT,³ since the saliva ferment cannot penetrate the *cellulose envelope* of the granules. BRASSE,⁴ however, asserts that diastase also attacks ungelatinised starch.

SOLERA⁵ has made similar experiments on the behaviour of different varieties of starch towards saliva. He found that there was a variation both in the *ratio* between the *weight* of starch employed and that of the sugar obtained, and also in the *time* in which equal quantities of sugar were produced; and established the fact that the varieties of starch which were most *rapidly* decomposed did not necessarily yield the most sugar, but that on the contrary *potato starch* with the greatest velocity of decomposition produced the smallest amount of sugar. Quantitative experiments have also been made by LEA⁶ and by KÜBEL,⁷ *inter alios*.

Whether the action is more or less energetic depends upon the relative amount of the ferment.

Saliva diastase, according to PASCHUTIN,⁸ cannot transform unlimited quantities of starch into sugar.

With a relatively small proportion of ferment, the higher products, *dextrins*, are formed to a preponderating extent (GRÜTZNER⁹).

A similar result is effected by a relatively high temperature.

Diastase which has been weakened by heat has not, according to BOURQUELOT,¹⁰ lost its power of producing the first decomposi-

¹ Paschutin, *Müller-Reichert's Arch.*, 1871.

² Hammarstén, *Virchow-Hirsch Jb.*, 1871, 95.

³ Bourquelot, *Comptes Rendus*, civ., 71.

⁴ Brasse, *Comptes Rendus*, c., 454. ⁵ Solera, *Maly's Jb.*, 235, 1878.

⁶ Lea, *Journ. of Physiol.*, xi., 234, 1890. ⁷ Kübel, *Pflüg. A.*, lxxvi., 276.

⁸ Paschutin, *Centr. med. Wiss.*, 273, 1871.

⁹ Grützner, *Pflüg. A.*, xii., 1876. ¹⁰ Bourquelot, *Comptes Rendus*, civ., 576.

tion products of starch (*vide supra*), which, on the contrary, are formed with the same rapidity. On the other hand, its further action is completely destroyed, so that no typical *end-products* are formed.

In new-born animals the saliva ferment is stated to exist only in the parotid gland, and there, too, only sparingly (BIDDER and SCHMIDT¹). ZWEIFEL² and KOROWIN,³ however, found that, as a rule, the *freshly extirpated parotid gland* possessed diastatic power.

Pathological saliva has been examined by ZWEIFEL² with reference to the ferment it contains. In thrush the ferment appears to be absent. In a case of *angina catarrhalis* it was found to be normal (SALKOWSKI⁴).

A diastatic ferment is not present in the saliva of all animals. It is found in man and herbivora (GRÜTZNER⁵). But it is not present in the submaxillary gland—*e.g.*, of rabbits (SCHIFF,⁶ GRÜTZNER,⁷ LANGLEY⁸). The mixed saliva of *rodents* (*rats*) is said to have the strongest action, then that of the *carnivora*; it is weakest in the case of the sheep and goat (ASTASCHEWSKI⁹). That of the horse has a very vigorous action (ELLENBERGER and HOFMEISTER¹⁰). KRUGENBERG¹¹ found it to be widely distributed among fishes, occurring in the saliva and mucous membrane of the mouth.

Pancreas Diastase (*Amylopsin*).¹²—The diastatic action of pancreatic juice was discovered by BOUCHARDAT and SANDRAS.¹³ It can be detected both in the secretion and in an infusion of the gland. Pancreas diastase is identical with, or very similar to, the saliva ferment (HAMMARSTÉN¹⁴).

Active extracts are obtained by the same methods as are employed in the preparation of the proteolytic pancreas ferment (*vide Trypsin*). They obviously also contain the other ferments. DANILEWSKI¹⁵ endeavoured to obtain a ferment which was only

¹ Bidder and Schmidt, *Verdauungssäfte*, 23, 1852.

² Zweifel, *Unters. üb. den Verdauungs-App. der Neugeb.*, Berlin, 1874.

³ Korowin, *Centralbl. med. Wiss.*, 261, 305, 1873.

⁴ Salkowski, *Virch. A.*, cix., 358, 1887.

⁵ Grützner, *Pflüg. A.*, xii., 285.

⁶ Schiff, *Leç. d. l. Phys. de la Digestion*, i., 204, 1867.

⁷ Grützner, *Pflüg. A.*, xvi., 105. ⁸ Langley, *Journ. of Physiol.*, i., 68.

⁹ Astaschewski, *Centralbl. med. Wiss.*, 531, 1877.

¹⁰ *Ellenberger and Hofmeister, *Arch. f. wiss. u. prakt. Thierheilkunde*, xii., 265, 1881.

¹¹ Krukenberg, *Unters. physiol. Inst. Heidelb.*, ii., 41, 389.

¹² Wingrave, *Lancet*, i., 1251, 1898.

¹³ Bouchardat and Sandras, *Comptes Rendus*, xx., 143, 1085.

¹⁴ Hammarstén, *Lehrb. phys. Ch.*, 262, 1895.

¹⁵ Danilewski, *Virch. A.*, xxv., 279.

diastatically active by means of precipitation with collodion; COHNHEIM¹ tried the same method which v. WITTICH² employed in the case of the saliva ferment, using *anhydrous* glycerin, which does not dissolve trypsin, after previous treatment with alcohol. Glycerin extracts were also employed by NASSE.³

PASCHUTIN⁴ asserts that, by means of hydrogen potassium arsenate, the diastatic ferment can be isolated almost alone from the pancreas, whilst solutions of other salts extract all the ferments. From permanent fistulas a liquid which is only diastatically active is frequently obtained.⁵

The resulting products are the same as in the case of the other diastases;⁶ glucose, too, is formed, which is to be attributed to the presence of *maltase*.

According to NASSE,⁷ it differs from the diastase of malt and of saliva in being affected differently by neutral salts.

Its activity is also destroyed at about 65° C. A quantitative investigation of its activity has been made—*e.g.*, by ROBERTS.⁸

GRÜTZNER⁹ found that the diastatic activity of the pancreas was weakest six hours after a meal, and strongest after fourteen hours. According to his experiments, sodium chloride does not interfere with the action of the diastase, unless present in considerable concentration (6 per cent.), but sodium carbonate is prejudicial even in a 0.05 per cent. solution. The strong restrictive action of the latter salt is confirmed by GANS.¹⁰ Pancreas diastase is said to be wanting in new-born animals (KOROWIN¹¹).

According to the experiments of ROBERTS¹² and FLORESCO,¹³ the pancreas of the pig has the greatest diastatic activity; whilst that of the ox, sheep, and dog, is much weaker in its action. HAMBURGER¹⁴ asserts that the pancreas of the dog is more active than that of the ox.

¹ Cohnheim, *Virch. A.*, xxviii., 251.

² v. Wittich, *Pflüg. A.*, ii., 196.

³ Nasse, *Pflüg. A.*, xiv., 473, 1877.

⁴ Paschutin, *Müller-Reichert's Arch.*, 382, 1873.

⁵ Gamgee, *loc. cit.*, 221.

⁶ v. Mering and Musculus, *Z. physiol. Ch.*, ii., 411. Hamburger, *Pflüg. A.*, lx., 560.

⁷ Nasse, *Pflüg. A.*, xi., 156.

⁸ Roberts, *Proc. Royal Soc.*, xxxii., 145.

⁹ Grützner, *Pflüg. A.*, xii., 292.

¹⁰ Gans, *Verh. Congr. innere Med.*, 449, 1896.

¹¹ Korowin, *Centralbl. med. Wiss.*, 261, 305, 1873.

¹² Roberts, Lumleian Lecture. Quoted by Gamgee, *loc. cit.*, 220.

¹³ Floresco, *C. R. Soc. Biol.*, 77, 1896.

¹⁴ Hamburger, *Pflüg. A.*, lx., 557.

Recently it has again been asserted, by CHLODOUNSKI and ŠULC,¹ that starch remains for the most part unaltered during the action of the pancreas, and also that *dextrins* and glucose, but *not* maltose, are the substances formed.

Diastase Zymogen.—Whilst it has been proved in the case of pepsin and trypsin (*q.v.*) that the glandular cells do not contain the active enzymes themselves, but a parent substance, a *zymogen*, this conclusion has not yet been established in the case of pancreas diastase, although it is probable.

LIVERSIDGE² first separated the diastatic enzyme from the pancreas; he then exposed the residue to the air and found that a fresh infusion again showed a strong diastatic action. If, then, a zymogen exists, it must be insoluble in water, since an increase in the activity through the influence of any agent has never been observed in aqueous solutions, as in the case of other enzymes.

According to the special observations which GOLDSCHMIDT³ made on the parotid saliva of the horse, it is also not yet possible to prove that there exists a zymogen of this, which is rendered active through the influence of the air.

The Diastatic Ferment of the Small Intestine.—Whilst, formerly, it was asserted that the mucous membrane of the small intestine contained no diastatic ferment (*e.g.*, by THIRY, LEUBE, LEHMANN⁴), it has at the present time been definitely proved that a decomposition of starch actually does take place in the intestinal secretions (GUMILEWSKI,⁵ LANNOIS and LÉPINE,⁶ EICHHORST,⁷ PASCHUTIN,⁸ DOBROSLAWIN⁹). BROWN and HERON,¹⁰ BASTANIELLI,¹¹ TEBB,¹² and others, detected *maltose* as a product of this decomposition. HAMBURGER¹³ ascribes very little diastatic power to the intestinal secretions; he found practically only *glucose*, as was also done by PREGL,¹⁴ though this undoubtedly is only formed from the maltose by a secondary

¹ Chlodounski and Šulc, *Sitzb. d. k. böhm. Acad. d. Wiss.*, 1896 (Bohemian). *Maly's Jb.*, 67, 1896.

² Liversidge, *Journ. of Anat. and Physiol.*, viii., 23, 1874.

³ Goldschmidt, *Z. physiol. Ch.*, x., 273.

⁴ Quoted by Röhmnn, *Pflüg. A.*, xli., 424 (gives the older literature).

⁵ Gumilewski, *Pflüg. A.*, xxxix., 564.

⁶ Lannois and Lepine, *Arch. d. Phys.*, 92, 1883.

⁷ Eichhorst, *Pflüg. A.*, iv., 584.

⁸ Paschutin, *Müller-Reichert's Arch.*, 1871.

⁹ Dobroslawin, *Unters. Anat. Phys. Inst. Graz*, 68, 1870.

¹⁰ Brown and Heron, *Lieb. Ann.*, cciv., 228.

¹¹ *Bastanielli, *Moleschott's Untersuch.*, 138, 1892.

¹² Tebb, *Journ. of Physiol.*, xv., 421, 1893.

¹³ Hamburger, *Pflüg. A.*, lx., 560. ¹⁴ Pregl, *Pflüg. A.*, lxi., 388.

reaction (*vide maltase*). RÖHMANN¹ found, like LANNOIS and LÉPINE, that the upper part of the small intestine acted more energetically than the lower. Both the intestinal secretion itself, and also the dried mucous membrane or glycerin extracts thereof, are active. On the other hand, according to GRÜTZNER,² Brunner's glands contain no diastase. GRÜNERT³ discovered diastase and invertase in the intestine.

A diastatic ferment was found in the intestine of the cray-fish by HOPPE-SEYLER,⁴ in that of bees by ERLÉNMEYER,⁵ and in the liver secretion of snails by KRUKENBERG⁶ and others.⁷

Liver Diastase.—The facts that the liver contains a carbohydrate similar to starch *glycogen*, that it forms this from the glucose of the blood, and that after death, and indeed also *intra vitam*, this substance is readily transformed into sugar, were made known notably by the investigations of CLAUDE BERNARD.⁸

Glycogen resembles starch, but possesses a still more complicated structure.⁹

It is converted into less complex sugar both by the action of acids and of *diastase*. In this process *maltose* is produced (KÜLZ and VOGEL.¹⁰)

It was thus natural to conclude that this decomposition in the liver was *also* effected by a *ferment* similar to diastase.

v. WITTICH¹¹ claims to have obtained a diastatic ferment from a glycerin extract of dried liver, as also from liver from which all blood has been completely drained, and hence he concludes that there is a *definite* liver ferment. He also found it in *bile*. In no case did he obtain these extracts free from sugar. SEEGEN and KRATSCHMER¹² by treating the livers of rabbits in as fresh a state as possible with glycerin, obtained extracts free from sugar. These contained the diastatic ferment and *glycogen*, the latter on dilution with water being transformed into sugar.

ABELES¹³ was the first to announce that the ferment was pro-

¹ Röhmann, *Pflüg. A.*, xli., 424. ² Grützner, *Pflüg. A.*, xii., 285.

³ Grünert, *Centralbl. f. Physiol.*, v., 285.

⁴ Hoppe-Seyler, *Pflüg. A.*, xiv., 394.

⁵ Erlenmeyer, *Münch. Acad. Sitzb., Math. Naturw. Cl.*, 205, 1875.

⁶ Krukenberg, *Unters. physiol. Inst. Heidelb.*, ii., 75, 411, 1878.

⁷ Cf. Biedermann and Moritz, *Pflüg. A.*, lxxiii., 247, 1898.

⁸ Cl. Bernard, *Comptes Rendus*, xli., 1855; lxxxv., 519, 1877.

⁹ Heine, *Fortschr. d. Medicin*, xiii., 789.

¹⁰ Külz and Vogel, *Z. f. Biol.*, xxxi., 108; cf. Musculus and v. Mering; *Z. physiol. Ch.*, ii., 416.

¹¹ v. Wittich, *Pflüg. A.*, vii., 28.

¹² Seegen and Kratschmer, *Pflüg. A.*, xiv., 593.

¹³ Abeles, *Med. Jahrbücher*, II. Heft, 1876, quoted by Schwiening.

duced after death, and that he had also obtained the ferment from *boiled livers*; this was confirmed by SEEGEN and KRATSCHMER,¹ though they refused to admit that it indicated a *post-mortem* product, inasmuch as they were able to detect the fermentation in the extract of boiled livers, even without any contact with the organ. They believed that they were justified in concluding that the diastatic reactions in these extracts were solely due to proteid substances—a conclusion similar to that arrived at by BARANETZKI² and MULDER.³ SCHWIENING⁴ cannot accept this conclusion. He is inclined rather to attribute the phenomenon to *bacterial action*, though he also is of opinion that the ferment is not completely destroyed on boiling, but only weakened. PAVY⁵ energetically supports the view that the decomposition of the glycogen in the liver is a fermentative process, and asserts that he has been able to preserve the ferment for a long period. This has been confirmed by TEBB.⁶ The whole question, therefore, still remains obscure, and stands in urgent need of elucidation.

Thus the question of the decomposition of glycogen *during life* is closely bound up with the question of the *diastatic ferment of the liver* and its action. The doubt arises, for instance, whether this decomposition is simply an *enzymic* one, like that of the proteids and carbohydrates in the intestine, or whether it is a specific *vital* process only effected by the living cells, and after removal of the organ merely continuing to a gradual extinction.

The ferment theory is accepted by SALKOWSKI⁷ and RICHEL,⁸ *inter alios*, whilst CAVAZZANI⁹ and PATON¹⁰ refuse to accept it. Salkowski concludes that an active *ferment* is present from the fact that the fresh liver decomposes glycogen when the protoplasm has been killed by chloroform. CAVAZZANI¹¹ urges against this apparently convincing proof that this might be due to the saccharifying action of the blood contained in the liver. He

¹ Seegen and Kratschmer, *loc. cit.*, 597.

² Baranetzki, *Die Stärkeumbildenden Fermente in d. Pflanzen*, Leipzig, 1878.

³ Mulder, *Chemie des Bieres*, translated by Grimm, Leipzig, 222, *et seq.*, 1858.

⁴ Schwiening, *Virch. A.*, cxxxvi., 465.

⁵ Pavy, *Journ. of Physiol.*, xx., 4 (Proc. Physiol. Soc. Oxford, 1896); xxii., 391, 1898.

⁶ Tebb, *Journ. of Physiol.*, xxii., 427, 1898.

⁷ Salkowski, *Pflüg. A.*, lvi., 339.

⁸ Richet, *C. R. Soc. Biol.*, 1894, 525.

⁹ Cavazzani, *Arch. Ital. d. Biol.*, xxviii., 91, 1898.

¹⁰ Paton, *Philosoph. Transact.*, clxxxv., 277, 1897.

¹¹ Cavazzani, *Arch. Ital. d. Biol.*, xxxii., 350, 1899.

firmly adheres to the vital view, and considers that he has a proof of it in the fact that when he disabled the cells with *methyle violet* instead of with chloroform he found no saccharifying action in the freshly-extirpated organ. Moreover, *quinine*, which has no action upon ferments, is said to considerably *lower* the saccharifying power of the liver, and the livers of dogs poisoned with quinine contain very little glucose, as CAVAZZANI¹ asserts in his last communication, in which he firmly maintains his opposition to the ferment theory. EVES,² too, considers that the formation of sugar by the liver is not of an enzymic nature. Inasmuch, however, as diastatic ferments occur everywhere in the body, it is not of such great importance whether this glycogen decomposition is more or less closely bound up with the living cells. From a theoretical point of view it is in any case a *fermentative process*.

Occurrence of Diastase in Other Organs and Secretions of Animals.—Diastatic ferments have been detected in most of the organs of the body, notably by v. WITTICH³ and by LÉPINE.⁴ In the horse they were found by ELLENBERGER and HOFMEISTER⁵ to be widely distributed. They were discovered in the crop, testicles, and thyroid glands, and also in the fourth stomach of the ox and other animals, by E. FISCHER and NIEBEL,⁶ in bile by JACOBSON,⁷ v. WITTICH,⁸ and others; in human milk by BECHAMPS,⁹ and in fæces by v. JAKSCH.¹⁰ As has long been known, a diastatic ferment is present in the *blood*.¹¹

PLÓSZ and TIEGEL¹² discovered a saccharifying ferment in combination with the blood-corpuscles, which is normally inactive, but through the influence of various agents (*e.g.*, freezing, &c.) becomes active. BIAL,¹³ on the other hand, found that the corpuscular elements were devoid of action.

PLÓSZ and TIEGEL discovered at the same time in the blood-corpuscles a force which destroyed ferments (possibly an oxydase?). They are inclined to attribute the diastatic action of the

¹ Cavazzani, *Arch. Ital. d. Biol.*, xxxii., 350, 1899.

² Eves, *Journ. of Physiol.*, v., 342. ³ v. Wittich, *Pflüg. A.*, vii., 28.

⁴ Lépine, *Sächs. Acad.*, 322, 1870.

⁵ Ellenberger and Hofmeister, *A. f. wissenschaft. Thierheilk.*, viii.

⁶ E. Fischer and Niebel, *Berl. Acad.*, v., 1896.

⁷ Jacobson, *De sacchari formatione fermentique, &c.*, Diss. Regimonti, 1865.

⁸ v. Wittich, *Pflüg. A.*, iii., 341.

⁹ Béchamp, *C. R.*, xvi., 1508.

¹⁰ v. Jaksch, *Z. physiol. Ch.*, xii.

¹¹ For the older literature, see Bial, *Pflüg. A.*, lii., 137.

¹² Plósz and Tiegel, *Pflüg. A.*, vi., 249; vii., 391.

¹³ Bial, *Pflüg. A.*, lii., 137; liii., 156.

liver (*vide supra*) to this blood diastase, which enters into combination with the proteids of the cells of the liver. v. WITTICH¹ denies both the combination with the blood-corpuscles and the explanation of the action of the liver. He also obtained it from the serum. BIAL² asserts that the blood contains a special ferment, which converts starch into *glucose*. He has thus overlooked the *successive* action of *diastase* and *maltase*. RÖHMANN³ discovered dextrins (*porphyro-dextrin* and *achroo-dextrin*), *iso-maltose*, and *glucose*. HAMBURGER⁴ found *maltose* also.

BIAL² further states that human blood has a *weaker* diastatic action; in the blood of new-born animals and of embryos he was at most able to detect diastase in traces.

ZANIER⁵ found more diastatic ferment in the blood of the mesenteric vein than in other vessels. It became weaker in starving animals.

Diastase was found in the serum of numerous animals by E. FISCHER and NIEBEL.⁶ In the *lymph* it was found by BIAL² and by RÖHMANN.⁷

LÉPINE⁸ states that the diastatic ferment in the blood is diminished in *diabetes*, as is also the case in long-continued *asphyxia*.

BÉCHAMP⁹ discovered a diastatic ferment in normal urine, which he regarded as a "matière albuminoïde" and named *nephrozymase*; he believed it to originate in the kidneys (see also COHNHEIM,¹⁰ BREUSING,¹¹ HOLOVTSCHINER¹²). GEHRIG¹³ found it in various urines, the least being in that of the dog.

In diabetic urine it was found by PLÓSZ and TIEGEL (*loc. cit.*); LÉPINE,¹⁴ however, found that it was present in smaller quantity in such urine.

A diastatic ferment was found in pleuritic exudations by EICHHORST,¹⁵ in the *cerebro-spinal liquid* (obtained by spinal

¹ v. Wittich, *Pflüg. A.*, vii., 28.

² Bial, *Pflüg. A.*, lii., 137; liii., 156.

³ Röhmnn, *Ber. d. d. chem. Ges.*, xxv., 3654. *C. med. Wiss.*, 849, 1893.

⁴ Hamburger, *Pflüg. A.*, lx., 570.

⁵ Zanier, *Gazzetta degli Ospitali*, 44, 1895. *Maly's Jb.*, xxvi., 212.

⁶ Fischer and Niebel, *Sitzb. Berl. Acad.*, v., 1896.

⁷ Röhmnn, *Pflüg. A.*, lii., 157 (older literature).

⁸ Lépine, *Wiener. med. Presse*, 1892, No. 26, &c. *Comptes Rendus*, cxiii., 1014, 1891.

⁹ Béchamp, *Comptes Rendus*, lx., 445, 1865.

¹⁰ Cohnheim, *Virch. A.*, xxviii., 251, 1865.

¹¹ Breusing, *Virch. A.*, cvii., 186, 1887.

¹² Holovtschiner, *Virch. A.*, civ., 42.

¹³ Gehrig, *Pflüg. A.*, xxxviii., 35.

¹⁴ Lépine, *loc. cit.*

¹⁵ Eichhorst, *Zeitsch. f. klin. Med.*, iii., 537, 1881.

puncture) by CAVAZZANI¹ and GROBER,² in dropsical fluid by BREUSING,³ and in hen's egg, by JOH. MÜLLER.⁴ PANZER⁵ discovered diastase in the liquid from a chyle fistula.

Diastatic Ferments and Diabetes.—An important part is ascribed to diastatic ferments in the etiology of *diabetes mellitus*.

Thus LÉPINE and BARRAL⁶ assert that, in *phloridzin diabetes*, the saccharifying ferment of the blood is increased, whilst in ordinary diabetes it is diminished (*vide supra*).

HILDEBRANDT⁷ first proved that the aqueous extract of *Syzygium Jambolanum*, *in vitro*, lowered the diastatic action, and then⁸ studied its influence on the artificial *diabetes* produced by irritating the *N. depressor*. He considers that the following conclusions can be drawn from his experiments:—*Syzygium Jambolanum* beyond doubt lowers the *amount of sugar separated*; but, inasmuch as its influence upon the *utilisation* of the sugar once formed must be regarded as very trifling or even *nil*, there is reason for concluding that its action causes a diminution in the *formation* of sugar, or, in other words, a weakening of the *diastatic power*.

He also found that arsenic injured, on the one hand, the action of diastase, and on the other hand, as was found by Salkowski, prevented the separation of sugar.

Finally, he found that injection of diastase lowered the limit of assimilation of grape sugar simultaneously introduced.

GANS⁹ has shown that, *in vitro*, the decomposition of glycogen by diastase can be retarded by adding sodium carbonate. He is inclined to conclude that the curative action of alkalies in *diabetes*, which has been clinically proved, depends upon this diminution in the *production of sugar* being also effected in the organism. This view, however, cannot be altogether accepted without further proof. On the one hand, Lépine explains the increase in the *excretion of sugar* by the decrease in the activity of the *glycolytic ferment* (*q.v.*), whilst, in his opinion, the *production of sugar* plays only a secondary part in the process; then, on the other hand, it has been assumed that in diabetes there is a diminution in the alkalinity of the blood, and the curative

¹ Cavazzani, *C. f. Phys.*, x., 145.

² Grober, *Münch. med. W.*, 247, 1900.

³ Breusing, *Virch. A.*, cvii., 186, 1887.

⁴ Müller, *Münch. med. W.*, 1583, 1899.

⁵ Panzer, *Z. physiol. Ch.*, xxx., 113, 1900.

⁶ Lépine and Barral, *C. R.*, cxiii., 1014, 1891.

⁷ Hildebrandt, *Berl. klin. W.*, No. 1, 1892.

⁸ Hildebrandt, *Virch. A.*, cxxxi., 26, 1893.

⁹ Gans, *Verh. Congr. innere Medicin*, 449, 1896.

action of alkalies is attributed to a restoration of this normal alkalinity.

Again, experiments have been made which indicate that a sort of *immunisation* against diastatic ferments can be produced by the introduction of *diastase* (*intravenal*), and through this a *decrease in the amount of sugar excreted* by diabetic patients can be brought about (KUSSMAUL,¹ LÉPINE and BARRAL²).

¹ Kussmaul, *A. f. klin. Med.*, xiv., 42, 1874.

² Lépine and Barral, *loc. cit.*

CHAPTER XVI.

FERMENTS OF POLYSACCHARIDES WHICH RESEMBLE
DIASTASE.

THE ENZYME WHICH DISSOLVES THE CELL-WALLS.

(CELLULASE, CYTASE.)

IN addition to reserve-starch the endosperm of many plants also contains considerable quantities of *reserve cellulose* or of similar substances which can be used in the formation of the cell-walls. For instance, in the case of *palms* the exterior wall is so enormously developed that the cavity appears to have nearly disappeared. Now these large quantities of cellulose are dissolved at the same time as the starch in the process of germination. This process of solution was first noticed by MITSCHERLICH¹ in sections of potato. SACHS² observed the solution of the endosperm and the production of *sugar*. Since then this process has frequently been further investigated, mainly from the microscopic-histological point of view.³ As GREEN⁴ shows, the notion of a dissolved enzyme forcibly suggested itself *a priori* in this process of solution, since for the histological reasons which he brings forward it is difficult to conceive in what manner the protoplasm of the *cotyledon* could act directly upon the material which is dissolved. Even the *production of granules* in the cells of the *haustorium*, into which part of the cotyledon is transformed, appears to indicate secretory activity. He did not, however, succeed in detecting any enzymes whatever in the extracts of these organs of palms.

On the other hand, BROWN & MORRIS⁵ found such enzymes in

¹ Mitscherlich, *Berliner Academ. Sitzb., Math. Naturw. Cl.*, 102, 1850.

² Sachs, *Bot. Ztg.*, 243, 1862.

³ *Vide* Reiss, *Landw. Jahrb. von Thiel*, xviii., 711, 1899, which contains copious references.

⁴ Green, *Annals of Botany*, vii., 93, 1893.

⁵ Brown and Morris, *Journ. of the Chem. Soc.*, lvii., 497, 1890.

germinating *barley corns*. They concluded that in the process of germination the cell-walls begin to dissolve before the starch granules are decomposed by the *diastase*. The epithelial cells of the *scutellum* also undergo a granular alteration in the process, which may be attributed to a state of secretion, similar to that described, notably by HABERLANDT,¹ in the case of diastase. They thus appear to secrete not only diastatic but also cytolytic enzymes.

By extraction with cold water and precipitation with alcohol, they obtained the enzyme in a dry condition (though not free from diastase), and when re-dissolved this was capable of dissolving the cell-walls of the barley-endosperm. The extract becomes inactive on boiling; at 60° C. it loses its cytohydrolytic power without being deprived of its diastatic capacity, which is first destroyed at 70° C. The enzyme acts best in a faintly acid solution, especially in one containing acetic or formic acid. Its chemical activity has as yet been very insufficiently investigated, and definite decomposition-products (sugar?) have not yet been isolated.

It also acts upon certain other cell-wall materials, though it is without action on many others, from which we must conclude that there are different substances in the walls of cells.

It is only produced when the nutritive substances in the cells diminish, as has also been shown to be the case with other vegetable enzymes (e.g., *invertase* in *Aspergillus*, *q.v.*). BEYERINCK² also comes to the conclusion that there is an enzymic solution of the reserve-cellulose by means of a special ferment. He has observed that even before being dissolved this is converted into a substance which gives the *blue* iodine reaction.

NEWCOMBE³ discovered a special *cytase* in the germinating endosperms of the *date*, *barley*, and *white lupine*, which were active in extracts; there was also a very faint action in the case of the *pea* and *buckwheat*. The cell-walls first became transparent, and finally dissolved. The various extracts behaved so differently towards starch and cellulose as to justify the conclusion of the existence of several enzymes. In particular, it was found that the *cytase* from the *endosperm of the date* acted decidedly more energetically upon *cellulose*, but had a much weaker action upon starch than the enzyme from the *cotyledons* of dates.

REINITZER,⁴ on the other hand, opposes the conclusion of a specific *cytase* peculiar to barley. BROWN and MORRIS had

¹ See p. 170.

² Beyerinck, *Centr. Bakt.* 1895, part 2, 239.

³ Newcombe, *Ann. of Bot.*, xiii., 49, 1899.

⁴ Reinitzer, *Z. physiol. Ch.*, xxiii, 175.

accounted for the fact of many cell-walls not being attacked by their enzymes by the *lignification* of these cells; REINITZER shows that this cannot invariably be the explanation. He concludes rather that the cell-walls of barley consist for the most part of easily-hydrolysable *hemi-celluloses*, which can be dissolved by even a 0.1 per cent. solution of hydrochloric acid; only these can be attacked by the enzyme, whilst there are other *hemi-celluloses* which remain absolutely unchanged. Moreover, whilst pure cellulose is not acted upon by an extract of air-dried malt, these readily hydrolysable *hemi-celluloses* are attacked by *diastase*—a power which the diastase, weakened by heating to 60° C., naturally no longer possesses to its full extent. Other *hemi-celluloses*, however, resist the action of diastase, and in the case of these Reinitzer admits the possibility of a specific enzyme, a *cytase*, which he absolutely refuses to recognise as present in *germinating barley*.

GRÜSS,¹ too, supports the view that it is the *diastase* which dissolves the cell-walls; he has studied this process microscopically in the case of the date, by immersing fine sections for a very long period in glycerin and preventing putrefaction by means of chloroform. He names the process of solution *alloolysis*. There result from it soluble products, presumably *mannose*.

Malt diastase and *Penicillium diastase* are also stated to have a similar action upon the reserve-cellulose (GRÜSS²).

A *gum ferment* which was found in certain plants by WIESNER³ is stated to possess the power of converting cellulose into gum or mucilage. REINITZER,⁴ however, denies that it possesses this property, and regards it as a simple diastatic ferment.

In like manner, there has been much discussion on the question of the occurrence of such *cytases* in *wood-destroying fungi* and similar parasites. Their activity has been closely studied, histologically and chemically,⁵ but the question of the enzyme is still waiting for a definite solution.

Experiments made by KOHNSTAMM,⁶ however, with the pressed extract of *Merulius lacrymans*, the *dry-rot*, obtained by Buchner's

¹ Grüss, *Ber. d. d. botan. Ges.*, xii., 1894.

² Grüss, *Festschr. f. Schwendener*, Berlin, 184, 1899.

³ Wiesner, *Sitz. Wiener Acad.*, xcii., 1, 40, 1886.

⁴ Reinitzer, *Z. ph. Ch.*, xiv., 453.

⁵ See also Hartig, *Die Zersetzungserscheinung. des Holzes*, Berlin, 1878. Also *Der Echte Hausschwamm*, Berlin, 1885; Wortmann, *Biol. Centralbl.*, iii., 285.

⁶ Ph. Kohnstamm (Munich). From a friendly private communication of the results of experiments not yet published.

method, seem to have furnished evidence of the existence of a true cellulose-dissolving enzyme. In the action of the *pressed extract* for fifty hours on the leaves of *Elodea*, he obtained results similar to those observed by HARTIG (*loc. cit.*) in the case of the *living fungus*—viz., *corrosions* in the form of large, narrow, extended *spots*, which caused the interior walls of the leaf to appear undulating and transversely striated in the form of a ladder.

Other processes of disintegration have been observed in the case of other *parasites*.

DE BARY¹ found that certain species of *Peziza* broke through and dissolved, by means of their mycelia, the central lamellæ of the plants attacked by them, that the juice expressed from these injured plants, dissolved cellulose, and that this property was lost on boiling.

In an analogous manner, MARSHALL WARD² observed the same phenomena of solution in the case of a parasite of the *lily*, a species of *Botrytis*, as with the *Peziza*; but, in addition to this, the mould-fungi, when grown in a nutrient liquid, formed secretions *containing granules* which gradually dissolved cellulose. The enzyme could be obtained in a dry condition by means of precipitation with alcohol. Similar results have been obtained by MANABU MIYOSHI³ with *Botrytis cinerea* and *Penicillium glaucum*. NEWCOMBE⁴ isolated from *Aspergillus Oryzæ* a *cytolytic enzyme* which, in an aqueous extract, had an energetic action upon cellulose, but a much weaker one upon starch.

A mould-fungus which grows on filter paper has been described by OMELIANSKI.⁵

Hadromase.—In the destruction of wood by mould-fungi—*e.g.*, the common *dry-rot*, *Merulius lacrymans*—CZAPEK⁶ concludes that, in the first place, the “ester” of the cellulose present in the wood, the compound of the latter with *hadromal*, is decomposed into its constituents, *cellulose* and *hadromal*, and that then the *cytase* brings about the further decomposition of the cellulose into soluble products. A similar action is ascribed to *Trametes*, *Polyporus*, *Agaricus*, *Pleurotus*, and *Armillaria*. CZAPEK succee dedin obtaining the active enzyme of *Pleurotus*

¹ De Bary, *Botan. Ztg.*, 415, 1886.

² Marshall Ward, *Ann. of Bot.*, ii., 1888.

³ Manabu Miyoshi, *Jahrb. wissenschaft. Bot.*, xxviii., 277, 1895. See also Ward, *Ann. of Bot.*, xii., 565, 1898.

⁴ Newcombe, *Ann. of Bot.*, xiii., 49, 1899.

⁵ Omelianski, *Comptes Rendus*, cxxi., 653, 1895.

⁶ Czapek, *Ber. d. deutsch. bot. Ges.*, xvii., 166, 1899.

pulmonarius and *Merulius lacrymans* in a solid and stable condition by expression and precipitation with alcohol. He named this enzyme *hadromase*, and regarded it as distinct from *cytase*, which, in addition to *diastase*, is a product of wood-destroying mould-fungi. He was inclined to group it with the enzymes which decompose glucosides. Until its chemical action has been more fully studied, I prefer, on practical grounds, to consider it as closely allied to *cytase*, without committing myself to this conclusion by doing so.

Bacteria, too, secrete enzymes which dissolve cell-walls—*e.g.*, *B. amylobacter* (DE BARY¹), *B. mesentericus vulgatus* (VIGNAL²), &c.

NÄGELI³ ascribes to bacteria in general the power of “converting cellulose into grape sugar.”

VAN SENUS⁴ obtained a *cytase* from putrefying beet-juice by precipitation with alcohol.

“*Cytases*” in Animals.—Most of the attempts to discover cellulose enzymes in *mammalia* have given negative results (DUCLAUX,⁵ PREGL⁶).

MACGILLAWRY,⁷ alone, claims to have obtained from the vermiform process of the rabbit a glycerin extract which digested cellulose, and to have been able to prepare from cellulose by means of this extract a substance which reduced copper oxide; SCHMULEWITSCH⁸ ascribes to the *pancreas* a solvent action on cellulose.

BROWN⁹ discovered a *cytase* in the intestine of graminivorous animals.

Cytases appear to be of more frequent occurrence in the *lower animals*.

In the case of fishes, KNAUTHE¹⁰ found that the extract of the *hepto-pancreas* of the *carp*, to which chloroform had been added, had a very energetic solvent action upon cellulose—*e.g.*, filter paper and the fruit of *Symphoricarpus racemosus* (snow-berries.)

BIEDERMANN and MORITZ¹¹ discovered in the middle intestinal gland (*liver*) of snails (*e.g.*, *Helix pomatia*) an extremely active

¹ De Bary, *Vorlesg. üb. Bacterien*, Leipzig, 65, 1866.

² Vignal, quoted by Green, *Ann. of Botany*, vii., 120.

³ Nägeli, *Die niederen Pilze*, 12, 1882.

⁴ Van Senus, quoted by Flüge, *Micro-organismen*, 207, 1896.

⁵ Duclaux, *Comptes Rendus*, xciv., 976, 1882.

⁶ Pregl, *Pflüg. A.*, lxi., 282.

⁷ MacGillawry, *Archiv. néerland.*, xi., 394, 1876.

⁸ Schmulewitsch, *Bull. Acad. St. Petersb.*, 549, 1879.

⁹ Brown, *Journ. Chem. Soc.*, lxi., 352, 1892.

¹⁰ Knauthe, *Zeitschr. f. Fischerei*, v., 1897.

¹¹ Biedermann and Moritz, *Pflüg. A.*, lxxiii., 236, 1898.

cytase, which in less than an hour commenced to dissolve both the endosperm of the date and still more resistant celluloses. An enzyme differing somewhat from this was found by the same investigators in the *liver* secretion of the *crayfish* (*loc. cit.*, p. 256). Both ferments decreased rapidly in their activity on dilution. *Extracts of the livers* were found to be inactive.

The enzyme produced *simple carbohydrates*—for instance, from *beet cellulose*, *glucose* (?) and a *pentose*; from *date stones*, *mannose* and *no pentose*. From the reserve-cellulose of the *coffee bean*, *mannose* and *galactose* were obtained; and, in short, the decomposition followed a course analogous to that effected by dilute acids.

The question of the alterations of cellulose in the *intestinal canal* and its physiological utilisation is, to a certain extent, connected with the solution of the cellulose by enzymes. It appears, however, as though we have to deal here almost exclusively with *putrefactive phenomena* and not with enzymic decompositions. I content myself, therefore, with referring to the note-worthy researches of TAPPEINER,¹ and the survey of the literature by BIEDERMANN and MORITZ.²

Inulase.—DRAGENDORFF³ was the first to assume the existence of an enzyme which transformed a carbohydrate, *inulin*, occurring in numerous organs of plants, into *fructose*, but he was unable to discover the ferment. It was subsequently found by GREEN⁴ in *Helianthus tuberosus*. BOURQUELOT⁵ discovered it in certain mould-fungi.

Where it occurs—*e.g.*, in *dahlias*, *artichokes*, &c.—inulin takes the place of the reserve-starch which is otherwise present. On decomposition it yields *d-fructose*. Malt contains *no* inulase. The inulase is produced in the organs of these plants on germination, but attempts to render the secretion histologically visible, as in the case of the diastase of plants, have not been successful. *It does not attack starch*, is destroyed by boiling, and is extremely sensitive to the action of acids.

It is present in the plants in the form of *zymogen* (GREEN⁶).

Seminase.—Diastatic ferments which convert *manno-galactan*, a reserve-substance of plants—the so-called *horn-albumin*—into

¹ Tappeiner, *Z. f. Biol.*, xx., 52, 1884.

² Biedermann and Moritz, *Pflüg. A.*, lxxiii., 219, 1898.

³ Dragendorff, *Materialien zu einer Monographie des Inulins*, St. Petersburg, 1870, quoted by Wortmann, *Biol. Ctbl.*, iii., 266.

⁴ Green, *Ann. of Bot.*, i., 223.

⁵ Bourquelot, *Bull. Soc. Mycol.*, x., 235. See also *ibid.*, ix., 230, reprint.

⁶ Green, *Ann. of Bot.*, vii., 122.

mannose and *galactose*, have been discovered by BOURQUELOT and HÉRISSEY¹ and others in the fruit of the carob tree, in species of *Medicago* and in *Trigonella fœnum græcum*. They conclude that a specific ferment, different from *diastase*, is here present, and have given it the name of *seminase*.

Carubinase.—The enzyme which is said to occur in the germinating seeds of *Ceratonia siliqua* has been as yet but little investigated, but is doubtless of a similar character, and has been named *carubinase* by its discoverer, EFFRONT.² By its action on *carubin*, a polysaccharide of which nothing further is known, there results a sugar which has been designated *carubinose*, but which, according to VAN EKENSTEIN,³ is identical with *d*-mannose.

Pectinase.—Under this name BOURQUELOT⁴ describes an enzyme occurring in germinated malt, which is capable of decomposing the *pectinous substances* resembling carbohydrates which are found in plants into reducing sugars, even when the pectins have been coagulated by their own accompanying enzyme, *pectase* (*q.v.*), though, *vice-versa*, pectase does not develop its activity *after the action of pectinase*. It occurs, together with *diastase*, in *malt*, but is absent (*e.g.*) from *saliva* and *aspergillus*, and in this respect differs from *diastase*.

It is very sensitive even towards a slight acidity in the media, which must, therefore, be rendered less acid by the addition of chalk before its action can be recognised.

¹ Bourquelot and Hérissé, *Comptes Rendus*, cxxix., 228, 391, 614, 1899; cxxx., 42, 340, 741, 1900. *Journ. d. Pharm. et Chim.* (6), xi., 104, 1900.

² Effront, *Comptes Rendus*, cxxv., 116, 1897.

³ van Ekenstein, *Comptes Rendus*, cxxv., 719, 1897.

⁴ Bourquelot, *Journ. d. Pharm. et Chim.*, 1899 (reprint). See also Bourquelot and Hérissé, *C. R. Soc. Biol.*, 777, 1898.

CHAPTER XVII.

FERMENTS OF THE DISACCHARIDES.—MALTASE.

MUSCULUS and GRUBER¹ discovered that, in diastatic fermentation, *grape sugar* was formed in addition to the main product, *maltose*; v. MERING² made the important discovery that it was not a *primary* product at all. Although *maltose* was fermented by yeast, he concluded that the *maltose* must *first be converted into glucose* before alcoholic fermentation could set in. He certainly could not accept this as a fermentation process.

CUISINIER³ next patented, in the year 1855, a process for the preparation of bread, which was claimed to contain a large proportion of a sugar, *cerealose*. He and his pupil GÉDULD⁴ then found that *glucose* was here produced by a special enzyme which they named *glucose*.

Almost simultaneously, BOURQUELOT⁵ furnished the proof that in every fermentation of *maltose*, even in the lactic acid, fermentative *glucose* was actually formed first. He crippled the alcohol-forming power of the yeast (by means of chloroform) and found that its power of decomposing *maltose* remained intact. He and HAMBURGER⁶ came to the conclusion that this decomposition was due to a special *enzyme*, which the latter also named *glucose*. E. FISCHER⁷ subsequently succeeded in definitely solving the question with the aid of the *osazone reaction*, by means of which he was able to directly identify the *glucose* produced.

There exists, therefore, a specific enzyme which decomposes *maltose* into two molecules of *glucose*, and which is best described by the name of *maltase*.

¹ Musculus and Gruber, *Z. physiol. Ch.*, ii., 182.

² v. Mering, *Z. physiol. Ch.*, v., 187.

³ Cuisinier, quoted verbatim by Beyerinck, *C. f. Bakt.* (II.), i. 329, 1895; cf. *Chem. Centbl.*, 614, 1886.

⁴ Géduld, *Wochenschr. f. Brauerei*, viii., 545, 1891.

⁵ Bourquelot, *Journ. de l'Anat. et Phys.*, xxii., 162, 1886; *Journ. de Pharm.*, 420, 1883.

⁶ Hamburger, *Pflüg. A.*, lx., 575.

⁷ E. Fischer, *Ber. d. d. chem. Ges.*, xxviii., 1433, 1895.

Maltase is widely distributed throughout both the vegetable and animal kingdoms, and usually accompanies the *diastatic ferments* (*q.v.*); its occurrence in *malt extract* is of primary importance. BEYERINCK (*loc. cit.*) has obtained an active preparation from decorticated and oil-freed *maize* by extraction with a dilute solution of tartaric acid (0.1 : 250.0) containing some alcohol, and precipitation with stronger alcohol.

As in the case of the diastases, slight differences also appear to occur between the maltases, so that E. FISCHER¹ concludes that there are numerous maltases. In particular, there appears to be a difference between malt maltase and yeast maltase. The former resists, to some extent, the action of alcohol, whilst the latter is very rapidly destroyed by it. They also differ in their sensitiveness to heat. Its occurrence in *cryptogams* is of special importance. BOURQUELOT² and his pupils have found it in many plants.

Nearly all the yeast-moulds contain maltase. It is, however, more firmly attached to them than is the *diastase*, so that fresh living yeast yields no maltase to the infusion when treated with water. It is necessary to dry the yeast first (*vide infra*).

In the *Taka diastase* of the yeast of *Aspergillus oryzae* (*vide p. 172*), a maltase was found in addition to *invertase* by KELLNER, MORI, and NAGAOKA.³ As a general rule, the maltase in aqueous extracts of dried yeasts is accompanied by *invertase*, with the exception of *S. octosporus*,⁴ which does not contain *invertase*. Maltase is absent from all the *lactose yeasts*, as also from *kephir granules*, which contain *lactase* instead; in addition to these, reference may be made to *S. marxianus*⁵ which contains only *invertase*, *S. apiculatus* which contains neither enzyme, and some other *saccharomycetes*.

According to E. Fischer's directions,⁶ it is obtained by washing beer yeast with water, drying it rapidly, and digesting it with water at 35° C. Fresh yeast yields no maltase to water, so that normally the hydrolysis which precedes the alcoholic fermentation must take place within the cell.

HILL⁷ has isolated it from dried yeast by extraction with sodium hydroxide solution (0.1 per cent.) and precipitation with

¹ E. Fischer, *Z. physiol. Ch.*, xxvi., 74, 1898.

² Bourquelot and others, *Bull. Soc. Mycol.*, ix., 230, Reprint. B. and Hérissé, *ibid.*, x., 235, Reprint.

³ Kellner, Mori, and Nagaoka, *Z. physiol. Ch.*, xiv., 305.

⁴ E. Fischer, *loc. cit.*, 77.

⁵ E. Fischer and Lindner, *Ber. d. d. chem. Ges.*, xxviii., 3037, 1895.

⁶ E. Fischer, *Z. physiol. Ch.*, xxvi., 75, 1898.

⁷ Hill, *Journ. Chem. Soc.*, lxxiii., 634, 1898.

alcohol. The *optimum* temperature for its activity is 40° C., whilst at 55° C. it is destroyed (LINTNER and KRÖBER¹).

In an aqueous solution it will only keep for a few days. Hill, however, was able to keep it for several months in sterilised and well-closed flasks without material alteration. It is apparently destroyed by alcohol, and is also injured by chloroform (LINTNER and KRÖBER,¹ cf. E. Fischer, *loc. cit.*, 75).

HÉRISSEY,² on the other hand, asserts that *aspergillus maltase* is not affected by chloroform.

The activity of this and the other ferments which decompose the disaccharides can best be studied after crippling the vital activity of the yeast cells, which would cause alcoholic fermentation, by the addition of about 1 per cent. of toluene (E. Fischer, *loc. cit.*, 75), not chloroform.

Maltase appears to possess the power of decomposing *dextrins*, not *starch*; at least, most investigators assign to maltase the property of decomposing dextrins in the *after-fermentation*, inasmuch as very little dextrin is found in the finished beer, but considerable quantities of sugar.

Animal Maltases.—The fact that, in the conversion of starch into sugar by animal secretions (saliva, secretions of the intestine, and pancreas), *grape sugar* is formed in addition to *maltose* was made known by v. MERING and MUSCULUS,³ KÜLZ,⁴ and others. v. MERING⁵ showed that maltose was decomposed by the saliva and the pancreas into glucose. BOURQUELOT⁶ and HAMBURGER⁷ inferred that a specific enzyme was present which converted the maltose into glucose. SHORE and TEBB⁸ investigated the decomposing action of many dried tissues on maltose. The small intestine of the pig had the strongest action. TEBB⁹ also found maltase in the liver, kidneys, &c. BOURQUELOT¹⁰ found more maltase in the small intestine of rabbits than in the pancreas, and chiefly in the middle of the intestine.

Maltase was found in the blood by DUBOURG,¹¹ by GLEY and

¹ Lintner and Kröber, *Ber. d. d. chem. Ges.*, xxviii., 1050, 1895.

² Hérissé, *C. R. Soc. Biol.*, 915, 1896.

³ v. Mering and Musculus, *Z. physiol. Ch.*, ii., 403.

⁴ Külz, *Pflüg. A.*, xxiv., 81, 1881. Külz and Vogel, *Z. f. Biol.*, xxxi., 108, 1894.

⁵ v. Mering, *Z. physiol. Ch.*, v., 190.

⁶ Bourquelot, *Journ. de l'Anat. et Phys.*, xxii., 200, 1886.

⁷ Hamburger, *Pflüg. A.*, lx., 575. Cf. Röhmman, *Ber. d. d. Chem. Ges.*, xxvii., 3252, 1894.

⁸ Shore and Tebb, *Journ. of Physiol.*, xiii., 19.

⁹ Tebb, *Journ. of Physiol.*, xv., 421.

¹⁰ Bourquelot, *Comptes Rendus*, xcvi., 1000, 1883.

¹¹ Dubourg, *Sur l'Amylase de l'Urine*, Thèse, Paris, 1889.

BOURQUELOT,¹ and by HAMBURGER.² TEBB,³ and FISCHER and NIEBEL,⁴ detected its occurrence in the serum of numerous animals.

A ferment (? maltase) which decomposes dextrin is present in the blood of the frog, but only in the spring and summer and not in the winter. At the latter period of the year, dextrin injected into the blood passes unchanged into the urine.⁵

Invertase, Sucrase.—It has long been known that yeast ferments cane sugar. DUMAS and BOULLAY⁶ showed, in 1828, that cane sugar must lose a molecule of water before fermentation. DUBRUNFAUT⁷ discovered (1830) that in this process it was transformed into non-crystallisable sugar. PERSOZ⁷ discovered the lævorotatory power of invert sugar, and BIOT⁷ the inversion by acids.

It was recognised at a very early period that the conversion of cane sugar was not effected by the typical activity of the yeast, but that a *special* ferment co-operates with it.

Cane sugar is decomposed by this enzyme into one molecule of dextrorotatory *d*-glucose and one molecule of lævorotatory *d*-fructose. Inasmuch as the rotation of the fructose to the left is stronger than that of the glucose to the right, the mixture (in equal parts) of the two sugars is lævorotatory, and is hence termed *invert sugar*.

BAUDRIMONT and DUBRUNFAUT⁸ called attention to this enzyme of yeast after occasional statements by DÖBEREINER and MITSCHERLICH. BERTHELOT,⁹ who was the first to prepare it in a dry condition by *precipitation with alcohol*, gave it the name of "*ferment inversif*," whilst BÉCHAMP¹⁰ called it *zymase*. BERNARD detected its occurrence in the intestinal secretion.

Invertase was prepared by many methods and further examined by LIEBIG,¹¹ BERTHELOT,¹² DONATH,¹³ and others. HOPPE-SEYLER¹⁴ attempted to isolate it by extraction with water after

¹ Gley and Bourquelot, *C. R. Soc. Biol.*, 247, 1895.

² Hamburger, *Pflüg. A.*, lx., 575. Cf. Röhmman, *Ber. d. d. chem. Ges.*, xxvii., 3253, 1884.

³ Tebb, *Journ. of Physiol.*, xv., 421.

⁴ Fischer and Niebel, *Sitzb. Berliner Acad.*, v., 1896, Reprint.

⁵ Quoted from Schützenberger, *loc. cit.*, 255, Note.

⁶ Dumas and Boullay, *Ann. Chim. Phys.*, xxxvii., 45.

⁷ Quoted by Pasteur, *Die Alkoholgährig.*, German translation by v. Griessmayer, Stuttgart, 1878 (2nd Ed.), 8.

⁸ Baudrimont and Dubrunfaut, quoted by Quevenne, *J. pr. Ch.*, xiv., 334.

⁹ Berthelot, *Comptes Rendus*, li., 980, 1860.

¹⁰ Quoted from Schützenberger, *loc. cit.*, 240.

¹¹ Liebig, in his *Annalen*, cliii., 1, 137. ¹² Berthelot, *C. R.*, li., 980, 1860.

¹³ Donath, *Ber. d. d. chem. Ges.*, viii., 795, 1875.

¹⁴ Hoppe-Seyler, *ibid.*, iv., 810, 1871.

killing the living cells with ether. BARTH¹ made use of Salkowski's method² to attain the same end, heating the dried yeast to 105° C., and then thoroughly studied the properties of the *invertase* which he obtained from this dry sterile yeast by extraction with water and precipitation with alcohol. *Invertase* was prepared by extraction with glycerin by GUNNING,³ and in considerable quantity by O'SULLIVAN and TOMPSON⁴ from the *liquid* yeast obtained by allowing pressed yeast to stand (for one to two months). Very active solutions have been obtained from pure cultivations of *Aspergillus niger* (*vide infra*). *Invertase* was found in several other yeasts by KALANTHAR,⁵ and in *mucor yeasts* by FITZ.⁶

Fruitless attempts to prepare it in a pure condition were made by LEA⁷ and WRÓBLEWSKI.⁸

It was then further investigated by OSBORNE.⁹ He let the yeast stand in contact with alcohol, extracted the *invertase* with chloroform-water, and purified it to some extent by precipitation with lead acetate and dialysis. As thus obtained, it has but little ash. It is not a *proteid substance*, but still contains carbohydrate. According to HARTLEY,¹⁰ it can also be distinguished from proteids by means of the spectroscope.

Invertase, like *maltase*, cannot be isolated from healthy fresh yeast cells, or at most only to a trifling extent.¹¹ In order to overcome the resistance of the cells and obtain the enzyme in an aqueous extract, it is necessary to have recourse to agents such as alcohol (LEA¹²), chloroform, or toluene (BOURQUELOT,¹³ E. FISCHER¹⁴); or the *cell-wall may be destroyed* by trituration with powdered glass (E. FISCHER¹⁴), or by continued *maceration* (POTTEVIN and NAPIAS¹⁵). This end is also attained by dry heat.

The various yeast *invertases* differ in certain properties,

¹ Barth, *ibid.*, xi., 474. Cf. also Nägeli, *Münch. Acad.*, 178, 1878.

² Salkowski, *C. med. Wiss.*, 606, 1877.

³ Gunning, *Ber. d. d. chem. Ges.*, v., 821, 1872.

⁴ O'Sullivan and Tompson, *Journ. Chem. Soc.*, lvii., 834, 1890.

⁵ Kalanthar, *Z. physiol. Ch.*, xxvi., 89, 1898.

⁶ Fitz, *Ber. d. d. chem. Ges.*, vi., 48, 1873; ix., 1352, 1876.

⁷ Lea, *Journ. of Physiology*, vi., 1885.

⁸ Wróblewski, *Ber. d. d. chem. Ges.*, xxxi., 1134, 1898.

⁹ Osborne, *Z. physiol. Ch.*, xxviii., 399, 1899.

¹⁰ Hartley, *Journ. Chem. Soc.*, li., 58, 1887.

¹¹ O'Sullivan, *Journ. Chem. Soc.*, lxi., 593, 1892.

¹² Lea, *Journ. of Physiol.*, vi., 1885.

¹³ Bourquelot, *C. R. Soc. Biol.*, 205, 1896.

¹⁴ E. Fischer, *Z. physiol. Ch.*, xxvi., 75, 1898.

¹⁵ Pottevin and Napias, *C. R. Soc. Biol.*, 237, 1898.

notably in their sensitiveness towards disturbing influences and in their *optimum temperature*. It is significant of the physiological importance of the enzymes that the invertase of *top-fermentation* yeast has an *optimum temperature* about 25° C. higher than that of *bottom-fermentation* yeast. Here we have a typical *adaptation* of the enzymes.

Yeast invertase also decomposes *melitriose* (raffinose), a trisaccharide of beet sugar, into *fructose* and *melibiose*,¹ and also a carbohydrate which occurs in *Gentiana lutea*, into *gentianose*.²

Nearly all yeasts contain *invertase*; most of them in addition to *maltase*, the lactose yeasts, in addition to *lactase*. Some—e.g., *S. Marxianus*—contain only *invertase*.³

On the other hand, *invertase* is absent from some yeasts—e.g., *S. apiculatus* (HANSEN⁴).

In aqueous solutions, *invertase* is, without doubt, the most sensitive of all the enzymes. Very dilute acids have, it is true, a stimulating effect upon the action of the ferment here, as in general; but the contrary effect is brought about by even a very slight amount of acid (FERNBACH). Oxalic acid is specially injurious.

It becomes inactive at fairly low temperatures in an aqueous solution, even at 45° to 50° C. after long-continued exposure (A. MAYER⁵). According to KJELDAHL,⁶ it is rapidly destroyed at 70° C., its optimum temperature being 53° to 56° C. Naturally solutions of cane sugar are used in these determinations.

It is protected to some extent by cane sugar, like all enzymes by their substrata (A. MAYER⁵). According to KJELDAHL,⁶ *alkalies* and *mercury salts* are also prejudicial; *light* is without influence, according to Mayer, and the same remark applies to *hydrocyanic acid* and to *boric acid* (BÉCHAMP⁷). It is destroyed by *pepsin* in a slightly acid solution (FALK⁸). It is also injured by alcohol (A. MAYER⁹).

DUCLAUX¹⁰ found that the restrictive influence of mercuric chloride was but slight, but that that of *potassium cyanide* was pronounced.

¹ E. Fischer, *Z. physiol. Ch.*, xxvi., 75, 1898.

² Bourquelot, *C. R. Soc. Biol.*, 237, 1898.

³ E. Fischer, *loc. cit.*

⁴ Hansen, quoted by Müller-Thurgau, *Landwirthsch. Jahrb.*, 795, 1885.

⁵ A. Mayer, *Z. ges. Brauw.*, 1892, 86; *Enzymologie*, 23.

⁶ Kjeldahl, *Z. ges. Brauw.*, 1881, 457.

⁷ Béchamp, *Comptes Rendus*, lxxv., 337.

⁸ Falk, *Du Bois Archiv. f. Physiol.*, 187, 1882.

⁹ Mayer, *Enzymologie*, Heidelberg, 13, 1882.

¹⁰ Duclaux, *Ann. Inst. Past.*, xi., 1897.

The conditions of the action of invertase have been studied by TAMMAN,¹ A. MAYER,² and MÜLLER-THURGAU,³ *inter alios*. The decomposition of the cane sugar increases *approximately* in proportion to the quantity of ferment. At higher temperatures it shows a rapid decrease in its activity, but even at a fairly low temperature (40° C.) it becomes weaker at a relatively rapid rate.

Müller-Thurgau also discovered that the curve of the quantities of cane sugar decomposed in definite periods of time was only a straight line at lower temperatures, but that, on the contrary, at higher temperatures, the quantities acted upon in equal periods of time decreased with the time, though obviously the absolute quantities *within* the same time were greater at higher temperatures; he expressed this in a series. The amounts decomposed in equal periods of time at the temperatures—

	0°,	10°,	20°,	30°,	40° C.
stand in the ratio of	9	: 19	: 36	: 63	: 73.

He attributes the gradual decrease in the incremental activity to the accumulation of the *decomposition-products*, which *interfere* with the action. Pure cane sugar does not prevent the action, even when present in a very strong degree of concentration.

A curious theory as to the nature of invertase is advanced by O'SULLIVAN and TOMPSON.⁴ They assume that invertase is decomposed into a homologous series of *seven* different *invertans*, which are distinguished from one another by their molecular weights and by their different proportion of nitrogen and different optical rotation. *α-invertan* is apparently identical with the yeast albuminoid, and is insoluble in water; the enzyme proper is *β-invertan*, the second in the series. All are complex proteid substances and dextro-rotatory. *η-invertan*, the last of the series, which contains 1 part of albuminoid to 18 parts of carbohydrate, is a constant constituent of the group. For further particulars reference must be made to the original. Their communication also gives a mass of other interesting particulars about invertase.

According to MÜLLER-THURGAU,⁵ it is no longer present in completely clarified *wine*; any inversion which still occurs then is to be attributed to the tartaric acid; the enzyme can be detected, however, in newer wines.

The invertase of yeasts and other mould-fungi differs considerably from that of other plants in many particulars, especially in the matter of sensitiveness.

As regards its occurrence in other cryptogams, it was first observed by BÉCHAMP⁶ in *mould-fungi*.

¹ Tamman, *Z. physiol. Ch.*, xvi. See General Part.

² A. Mayer, *loc. cit.*

³ Müller-Thurgau, *Thiel's Landw. Jb.*, 795, 1885.

⁴ O'Sullivan and Tompson, *Journ. Chem. Soc.*, lvii., 834, 1890.

⁵ Müller-Thurgau, *loc. cit.*, 815.

⁶ Béchamp, *Comptes Rendus*, xlvi., 44, 1858.

Invertase was found by WASSERZUG¹ in fungi of the genus *Fusarium*, where it is produced during the formation of conidia; it is present, according to ZOPF,² in *Leuconostoc mesenteroides*, a parasite of beetroot juice, whilst GAYON³ found it in *Aspergillus*, but not in *Mucor*.

KOSSMANN⁴ was the first to discover it in many other mould-fungi and algæ belonging to the cryptogams. *Invertase* was next found by BOURQUELOT⁵ in many mould-fungi—e.g., in *Aspergillus*, but not in *Polyporus*. FERNBACH⁶ has studied the invertase of *Aspergillus* with reference to its physiological significance. He found that it was only present when the mould-fungi began to attack their reserve-material—i.e., when they had *need* of it. In order to express its quantity in comparable figures, he has devised a "unit." The unit of "*sucrase*" is the quantity which inverts 20 centigrammes of saccharose at 56° C. *Aspergillus oryzae*, which secretes *Taka-diastase*, also contains invertase. *Aspergillus* invertase is much less sensitive to the action of acids.

The case of *Monilia candida* is interesting. Although it is capable of fermenting cane sugar, no invertase can be obtained from it. It thus appeared that there was here the only instance of a direct fermentation of cane sugar. E. FISCHER and LINDNER,⁷ however, succeeded in proving that the dried yeast *inverts cane sugar* when its characteristic vital alcohol-producing activity has been destroyed by means of toluene, and that the same result is obtained in the case of the fresh yeast by rupturing its cells with powdered glass. Hence, in the case of *Monilia candida* also, *hydrolysis* first occurs, and then alcoholic fermentation, but the specific invertase is apparently insoluble in water. FERNBACH⁸ also observed very similar phenomena in the case of other mould-fungi.

Individual bacteria also produce invertase, such as *B. mesentericus vulgaris* (VIGNAL⁹), *B. megaterium* (FERMI¹⁰), and certain others; some, however, only in an acid culture medium, others

¹ Wasserzug, *Ann. Inst. Pasteur*, i., 525, 1886.

² Zopf, quoted by Schlesinger, *Virch. A.*, cxxv., 156.

³ Gayon, *Comptes Rendus*, lxxxvi., 52, 1878.

⁴ Kossmann, *Bull. Soc. Chim.*, xxvii., 251, 1877.

⁵ Bourquelot, *loc. cit.*

⁶ Fernbach, *Ann. Inst. Pasteur*, iv., 1, 1890.

⁷ E. Fischer and Lindner, *Ber. d. d. chem. Ges.*, xxviii., 3034. *Z. physiol. Ch.*, xxvi., 77, 1898.

⁸ Fernbach, *loc. cit.*

⁹ Quoted by Green, *Ann. of Bot.*, vii., 120.

¹⁰ Fermi, *C. f. Bakt.*, xii., 713.

in an alkaline one. It also occurs occasionally in the *Cholera vibrio*.¹

Invertase of Phanerogams.—Higher plants, too, contain *invertase* in their living cells. That this enzyme plays a part in the economy of plants is manifest from the fact that they form cane sugar in their sap and *utilise* it, whilst, on the other hand, the cane sugar must be *inverted* before the assimilation, which cannot be effected by the acids of the plants alone.² It was found in *malt extract* by BROWN and HERON,³ in leaves by KOSSMANN,⁴ and in pollen-grains by VAN TIEGHEM.⁵

O'SULLIVAN⁶ then detected *invertase* in the organs of the *gramineæ* in the same way in which its presence was demonstrated in living yeast cells. He treated the roots, stalks, and leaves of wheat, peas, and maize, at about 50° C., with a solution of cane sugar saturated with chloroform. It was possible to detect and measure the *decomposition* which occurred in this process.

Animal Invertase.—In the animal organism, *invertase* occurs in the intestinal secretion, the inverting capacity of which was discovered by CLAUDE BERNARD.⁷ It has since then been frequently further investigated (RÖHMANN,⁸ v. MERING,⁹ GRÜNERT,¹⁰ MIURA,¹¹ KRÜGER¹²); it is also found in still-born animals, and is hence not absolutely dependent on the introduction with food (MIURA¹¹); it is absent from the intestine of the ox (EMIL FISCHER and NIEBEL¹³). In the upper part of the intestine it is found more than in the lower (Röhmman, *loc. cit.*).

On the other hand, it is not present in the *pancreas* and *saliva* (v. MERING,⁹ BROWN and HERON¹⁴), but occurs in the *saliva of bees* (ERLENMEYER¹⁵). It is also wanting in blood, since cane

¹ Fermi and Montisano, *C. f. Bakt.* (II.), i., 482, 542, 1895.

² Wortmann, *Biolog. Centralbl.*, iii., 263.

³ Brown and Heron, *Journ. Chem. Soc.*, xxxv., 609, 1879.

⁴ Kossmann, *Comptes Rendus*, lxxxii., 406.

⁵ van Tieghem, *Bull. Soc. Bot. d. France*, xxxiii., 216, 1886.

⁶ O'Sullivan, *Proc. Chem. Soc.*, xvi., 61. *Chem. Centralbl.*, i., 773, 1900.

⁷ Claude Bernard, *Leç. sur le Diabète*, Paris, 259, 1887.

⁸ Röhmman, *Pflüg. A.*, xli., 432 (gives the older literature). See also Köbner, *Z. f. Biol.*, xxxiii., 404, 1896.

⁹ v. Mering, *Z. physiol. Ch.*, v., 192.

¹⁰ Grünert, *C. f. Phys.*, v., 285.

¹¹ Miura, *Z. f. Biol.*, xxxii., 277.

¹² Krüger, *Z. f. Biol.*, xxxvii., 229, 1899.

¹³ Fischer and Niebel, *Sitzb. Berl. Acad.*, v., 1896, Reprint.

¹⁴ Brown and Heron, *Liebig's Ann.*, cciv., 288.

¹⁵ Erlenmeyer, *Münch. Acad. Sitzb., Math. Phys. Cl.*, 205, 1874.

sugar when injected into a vein appears unaltered in the urine (CL. BERNARD)¹.

RENZI,² however, found that, after extirpation of the salivary glands, the limit of the assimilation of cane sugar was lowered—a fact which urgently requires explanation.

ROBERTSON³ discovered invertase in nearly every organ.

According to NASSE,⁴ the action of the invertase is influenced by oxygen and carbon monoxide, though, according to FERMI and PERNOSI,⁵ hydrogen sulphide has no effect upon it. It is extremely sensitive towards *acids* and *alkalies*—i.e., at higher temperatures. It is also rapidly destroyed at 75° C. in the absence of acids.⁶ Neutral salts differ extraordinarily in their behaviour towards it, *ammonium salts* having a very pronounced stimulating effect, *potassium chloride* a strong restrictive influence, &c.⁷ *Alcohol* is *slightly* injurious, whilst *tartaric acid* has a favourable action.⁸

Trehalase.—An enzyme which decomposes *trehalose*, a disaccharide occurring in mould-fungi and a kind of manna (trehala) into *two molecules of glucose*, was discovered by BOURQUELOT⁹ in *Aspergillus* and other moulds, and also in green malt. He named it *trehalase*.

E. FISCHER¹⁰ found it in *green malt diastase*, and also, to a very trifling extent, in *yeasts of the Froberg type*, whence, of course, it does not pass into the aqueous extract. It was found in several other yeasts by ANNUSCH KALANTHAR.¹¹ BAU¹² proved that the different yeasts varied so irregularly in regard to the trehalase they contained that it was impossible to classify them on that basis.

Bourquelot (*loc. cit.*) obtained it from the aqueous extract by precipitation with alcohol. Very dilute acids further its action somewhat, but it is weakened by even slight quantities.

Its limit of activity is 64° C., by means of which BOURQUELOT¹³

¹ Cl. Bernard, quoted by Schützenberger, *loc. cit.*, 259.

² Renzi, *Berl. klin. Woch.*, No. 23, 1892.

³ Robertson, *Edinburgh Med. Journ.*, 1894, quoted by Edmunds, *Journ. of Physiol.*, xix., 466, 1895.

⁴ Nasse, *Pflüg. A.*, xv., 471.

⁵ Fermi and Pernossi, *Z. f. Hygiene*, xviii., 83.

⁶ See, *inter alios*, Green, *Annals of Botany*, vii., 92.

⁷ Nasse, *Pflüg. A.*, xv., 471.

⁸ Müller-Thurgau, *Thiel's Landwirthsch. Jahrb.*, 795, 1885.

⁹ Bourquelot, *Bull. Soc. Mycol. d. France*, ix., 64, 189, 1892, Reprint.

¹⁰ E. Fischer, *Z. physiol. Ch.*, xxvi., 79, 1898.

¹¹ Kalanthar, *Z. physiol. Ch.*, xxvi., 97, 1898.

¹² Bau, *Z. f. Spirit. Ind.*, 232, 1899, through *Chem. Centralbl.*

¹³ Bourquelot, *C. R. de la Soc. Biol.*, 425, 1893.

was able to separate it from maltase, which withstands a somewhat higher temperature.

Its existence cannot be demonstrated with absolute certainty, although the significant structural difference of maltose and trehalose renders conceivable the assumption that trehalase is only a modification of maltase (E. Fischer, *loc. cit.*, 81).

BOURQUELOT and GLEY¹ bring forward, in support of the specific nature of *trehalase*, the fact that blood serum which contains maltase does not attack trehalose.

There is as little proof of the specific character of the enzyme which converts *melicitose* into *turanose* and *glucose* (BOURQUELOT and HÉRISSEY²). They discovered it in *Aspergillus niger*.

Melibiose.—A characteristic *disaccharide*, *melibiose*, can be formed from *melitriose* or *raffinose* by the action of *invertase*, and this, on further decomposition, is split up into *d-galactose* and *d-glucose* in an analogous manner to *lactose*.

This decomposition is also effected by an enzyme which is present in some *bottom-fermentation yeasts*, but is absent from all *top-fermentation yeasts*. It acts both in the fresh and dried yeast, but only passes to a slight extent into an aqueous extract (E. FISCHER and LINDNER³). BAUER⁴ has given the name of *melibiase* to this enzyme. E. FISCHER,⁵ however, inclines to the view that *melibiase* is a somewhat aberrant *maltase*. *Invertase* does not act upon *melibiose*. The *adaptation* of yeasts to *melibiose* and the production of *melibiase* which results from it have been investigated by DIENERT.⁶

Lactase is, in like manner, an enzyme with a specific action, which confines its activity exclusively to milk sugar (*lactose*), and decomposes this *disaccharide* into *d-glucose* and *d-galactose*.

Its presence in milk-sugar yeasts (*S. kefir* and *S. tyrocola*) was first inferred by BEYERINCK,⁷ who gave it its name and then endeavoured to prove its existence by the fact that phosphorescent bacteria were only able to develop their activity on *lactose* culture-media when a certain amount of cultivations of the above-mentioned yeasts had been added to these culture-media. Some *glucose*, a food suited to these bacteria, was then formed (*auxanographic method, vide supra*). The value of these

¹ Bourquelot and Gley, *C. R. Soc. Biol.*, xlvii., 515, 1895.

² Bourquelot and Hérissé, *Comptes Rendus*, cxxv., 116, 1897.

³ E. Fischer and Lindner, *Ber. d. d. chem. Ges.*, xxviii., 3034, 1895.

⁴ Bauer, *Chemikerzeitung*, 1873, 1895.

⁵ E. Fischer, *Z. physiol. Ch.*, xxvi., 81, 1890.

⁶ Dienert, *Comptes Rendus*, cxxix., 63, 1899.

⁷ Beyerinck, *Centralbl. f. Bakteriöl.*, vi., 44, 1889.

experiments as a proof was, however, questioned by STEKHOVEN.¹ EMIL FISCHER² was the first to demonstrate its existence by proving the decomposition by means of the formation of *glucosazone*. He was able to prepare it from kephir granules by extraction with water and precipitation with alcohol, though, of course, in a condition not free from *invertase*. Although pure cultivations of milk sugar yeasts yielded but little enzyme soluble in water, the yeasts themselves energetically hydrolysed milk sugar in the presence of toluene, and thus behaved in a similar manner to *Monilia candida* in the case of *invertase* (*q.v.*). DIENERT³ has recently obtained *lactase* by triturating with distilled water yeasts which he had adapted to the fermentation of lactose.

Maltase and lactase appear to belong to distinct kinds of yeast, since they have never been associated in the same yeast (E. FISCHER²). Moreover, no lactose was found by BOURQUELOT and HÉRISSEY⁴ in mould-fungi which contained maltase. Lactase appears to be much more closely allied to ferments of the *emulsin* type (*vide infra*) than to other yeast enzymes of the *maltase* type, since *emulsin* also decomposes lactose, whilst *maltase* does not.

On the other hand, LABORDE⁵ has described in *Eurotiosis Gayoni* a mould which attacks both *maltose* and *lactose*, and thus appears to produce *lactase* also.

NÄGELI⁶ has also ascribed to bacteria the power of secreting a ferment which decomposes lactose.

No lactase was found by PREGL⁷ in the *intestinal* secretion.

The same result was obtained by PANTZ and VOGEL,⁸ and DASTRE,⁹ who were unable to discover lactase either in the pancreas or in the liver and intestinal secretion. On the other hand, RÖHMANN and LAPPE¹⁰ found *lactase* in the intestine of the calf and dog, but not in that of the ox.

PORTIER¹¹ confirmed and extended Röhmann's results; the

¹ Stekhoven, quoted by Fischer. See next reference.

² E. Fischer, *Z. physiol. Ch.*, xxvi., 81.

³ Dienert, *Comptes Rendus*, cxxix., 63, 1899.

⁴ Bourquelot and Hérissé, *Comptes Rendus*, 693, 1895; *Bull. Soc. Mycol.*, x., 235, Reprint.

⁵ Laborde, *Ann. Inst. Pasteur*, xi., 1, 1897.

⁶ Nägeli, *Die niederen Pilze*, 12, 1882.

⁷ Pregl, *Pflüg. A.*, lxi., 382.

⁸ Pantz and Vogel, *Z. f. Biol.*, xxxii., 304, 1895.

⁹ Dastre, *Archives d. Physiol.*, 103, 1890.

¹⁰ Röhmann and Lappe, *Ber. d. d. chem. Ges.*, xxviii., 2506, 1895.

¹¹ Portier, *C. R. Soc. Biol.*, 387, 1898.

pancreas, according to him, *never* contains *lactase*; he found it, however, in the intestine of *young* animals, and to a less extent in that of *full-grown* animals; in the case of old animals it was wanting, and also in birds. On the other hand, WEINLAND¹ was able to detect it in the *pancreas of the dog*, especially after a milk diet.

¹ Weinland, *Z. f. Biol.*, xxxviii., 606; *Chem. Centralbl.*, i., 1002, 1899.

CHAPTER XVIII.

FERMENTS WHICH DECOMPOSE GLUCOSIDES.

IF we take a comprehensive view of the various facts described in the preceding pages, we find that yeasts of the ordinary type undoubtedly contain, in addition to diastatic ferments, *two kinds of enzymes*, of which one variety, *maltase* and its relations, decomposes maltose and disaccharides of similar configuration, whilst *invertase* decomposes cane sugar and raffinose.

Possibly in addition to these there is a third independent variety—*trehalase*. In milk-sugar yeasts *lactase*, which has a totally different action, is found instead of *maltase*, but has unfortunately been but little investigated in a condition free from the influence of other enzymes.

EMIL FISCHER¹ attributes the specific activity of enzymes to stereo-chemical conditions, assuming that not only differences of structure, but also differences of *configuration* form the basis on which the specific activity of the enzymes depends. Of special significance for this point of view are his investigations on the action of enzymes on *glucosides*, not only on those which occur naturally, but also, in particular, on the *simple glucosides* synthetically prepared by him.

By the condensation of simple hexoses (glucose, mannose, sorbose, fructose, &c.) with alcohols, especially methyl alcohol, under the influence of hydrochloric acid, he succeeded in preparing artificially, *e.g.*, *methyl glucosides* of these sugars. In this condensation two *stereo-isomers* are invariably formed, which Fischer distinguishes as α - and β -glucosides—*e.g.*, α -methylmannoside and β -methylmannoside from *d*-mannose, &c. If now the behaviour of these simple glucosides towards *yeast enzymes* be tried, it will be found that the α -modification alone is decomposed into the original sugar, whilst the β -modification *absolutely resists* the action of the yeast enzyme.

From this it follows with certainty that the action of enzymes depends upon differences in the stereo-chemical structure of the

¹ E. Fischer, *Z. physiol. Ch.*, xxvi., 61.

glucosides. This receives further support from the fact that the glucosides of *non-fermentable sugars*—i.e., of all the *pentoses* and *heptoses*, as also of the whole of the *l-sugars* (e.g., *l-glucose*)—resist the action of yeast enzymes.

But whilst these partially structurally and partially stereo-chemically different glucosides are *absolutely* unattacked by any known enzymes whatever, the β -glucosides of fermentable sugars, which resist the action of *yeast-enzymes*, possess the property of being decomposed into their components by another ferment—viz., *emulsin*, which we shall subsequently discuss more fully. The β -glucosides share this property with a large number of *naturally-occurring glucosides*.

Now, of all *these* glucosides, only *one*, so far as is known, is attacked by yeast enzymes—viz., the *amygdalin* of bitter almonds. *Amygdalin* is decomposed by *emulsin* into *benzaldehyde*, *hydrocyanic acid*, and *grape sugar*. On the other hand, an *infusion of yeast* only splits off one molecule of *grape sugar*, whilst the remainder of the glucoside now consists of a characteristic substance, *almond nitrile glucoside*,¹ which resists the action of yeast enzymes, but is decomposed further by *emulsin* into *benzaldehyde*, *hydrocyanic acid*, and *grape sugar*. All the rest of the naturally-occurring glucosides, however, are absolutely unaffected by yeast enzymes, although they are partially decomposed by *emulsin*. In this respect they thus completely correspond with Fischer's β -glucosides.

By yeast enzymes, we understand here mainly *maltase* and *invertase*; *trehalase* has not been sufficiently examined, though it is natural to regard it as related to the yeast enzymes mentioned above, since *trehalose* is not changed by *emulsin* (BOURQUELOT²).

We see, then, that for the complicated derivatives of the sugars there are *two series of ferments completely differing in their activity*, the differences of which rest upon a stereo-chemical basis; their activity is most easily determined on the two stereo-isomeric series of α - and β -glucosides. I should like, therefore, to adopt the plan of simply distinguishing the two series as α - and β -ferments. We should then have the following groups:—

1. α -Ferments—

Invertase.

Maltases (including *melibiase*).

Trehalase (?).

¹ E. Fischer, *Ber. d. d. chem. Ges.*, xxviii., 1508, 1895.

² Bourquelot, *Bull. Soc. Mycolog.*, ix., 189, Reprint.

2. β -Ferments—

Emulsin.

Lactase (?).

Naturally, the remainder of the ferments which decompose glucosides are to be classified with *emulsin*.

Emulsin.—This enzyme, formerly known also as *synaptase*, decomposes the glucoside *amygdalin*, found in almond kernels, into *grape sugar*, *benzaldehyde*, and *hydrocyanic acid*.

Amygdalin was discovered and prepared in 1830 by ROBQUET and BOUTRON-CHARLARD.¹ They also described the property possessed by bitter almonds of liberating hydrocyanic acid and a substance with an aromatic odour on contact with water.

LIEBIG and WÖHLER² then (1837) thoroughly investigated amygdalin, and gave to the substance resembling albumin, which effects its decomposition into grape sugar, benzaldehyde, and hydrocyanic acid, the name *emulsin*.

ROBQUET³ afterwards described a non-coagulating substance precipitated by tannin as the active principle, which he termed *synaptase*.

Occurrence of Emulsin.—Emulsin, which Liebig and Wöhler were only able to discover in almonds, is widely distributed both throughout higher plants and among the cryptogams; the emulsins of these different groups of plants, however, appear to be different (HÉRISSEY⁴).

In phanerogams, in addition to its occurrence in almonds, *emulsin* is found above all in the leaves of *Laurocerasus* and in the seeds of various *Rosaceæ* (LUTZ⁵). In these plants occur glucosides resembling *amygdalin*—e.g., *laurocerasin* (to mention one of many), which are decomposed in an analogous manner by the *emulsin* simultaneously present in the plants. Emulsin also decomposes other glucosides—e.g., *arbutin*, *salicin*, *coniferin*, *populin* (*vide infra*). According to POLECK,⁶ *Schleichera trijuga*, from which is obtained *macassar oil*, which contains *hydrocyanic acid*, also produces hydrocyanic acid and benzaldehyde. *Amygdalin* also decomposes the extracts of numerous plants

¹ Robiquet and Boutron-Charlard, *Ann. Chim. Phys.* (2), xlv., 352, 1830.

² Liebig and Wöhler, *Ann. d. Pharm.*, xxii., 1, 1837. *Poggendorff's Ann.*, xli., 345.

³ Robiquet, *Journ. d. Pharm. et Chim.*, xxiv., 196, 1838.

⁴ Hérissé, *Recherches sur l'Emulsine*, Paris, 6, 1899.

⁵ Lutz, *Bull. Soc. Bot. d. France*, xlv., 26, 263, 1897. Quoted by Hérissé, *loc. cit.*

⁶ Poleck, *Pharmac. Ztg.*, 314, 1891.

Monotropa, *Polygala* (BOURQUELOT¹), *Isatis alpina* (BRÉAUDAT²), and a very large number of others, including *Malus communis*, *Hedera helix*, &c. (HÉRISSEY³). Many other plants yield, on distillation with water *only*, hydrocyanic acid, which appears to be formed by decomposition from glucosides (JORISSEN⁴)—*e.g.*, *Aquilegia vulgaris*, *Ribes aureum*, and *Manihot utilissima* (PFEFFER⁵).

In linseed, JORISSEN and HAIRS⁶ have discovered a special glucoside, which they name *linamarin*. It is decomposed by a *special* emulsin into hydrocyanic acid, a fermentable sugar, and a body of a ketonic character. The emulsin of almonds has no action upon this glucoside, although, conversely, an extract of linseed decomposes amygdalin (?). (Hérisséy, *loc. cit.*, 25.)

In cryptogams, emulsin was discovered by BOURQUELOT,⁷ who found it in *Aspergillus niger*. Simultaneously it was found by GERARD⁸ in *Penicillium glaucum*. BOURQUELOT⁹ was able to detect it in many moulds, notably those *growing on wood*; the investigation was subsequently continued by HÉRISSEY.¹⁰ Nearly all the parasitic moulds of the most widely different species examined decomposed amygdalin.

HÉRISSEY also found it in *mosses*.¹¹

Bacteria, too, are stated to contain ferments resembling emulsin; they decompose amygdalin with the formation of *benzaldehyde*, though glucose cannot be detected (GÉRARD,¹² FERMI and MONTISANO¹³).

Ferments of the nature of emulsin also appear to occur in the animal kingdom.

According to MORIGGIA and OSSI¹⁴ amygdalin has a toxic action, they account for this by the assumption that a fermentative decomposition occurs in the intestinal canal; FUBINI¹⁵ confirmed their assertion.

¹ Bourquelot, *Journ. d. Pharm. et Chim.* (5), xxx., 433, 1894.

² Bréaudat, *Bull. Soc. Biol.* (10), v., 1031, 1898.

³ Hérisséy, *loc. cit.*, 22, *et seq.*

⁴ Jorissen, *Journ. d. Pharm. d'Anvers*, 23, 1894.

⁵ Pfeffer, *Pflanzenphysiologie*, Leipzig, 307, 1881.

⁶ Jorissen and Hairs, *Bull. Acad. Belg.* [9], xxi., 518, 1891.

⁷ Bourquelot, *C. R. Soc. Biol.*, 653, 804, 1893.

⁸ Gérard, *ibid.*, 651, 1893.

⁹ Bourquelot, *Bull. Soc. Mycol.*, x., 49, 1894, Reprint.

¹⁰ Hérisséy, *loc. cit.*, 8, *et seq.*

¹¹ Hérisséy, *C. R. Soc. Biol.*, 532, 1898. *Bull. Soc. Mycol.*, xv., Reprint.

¹² Gérard, *Journ. Pharm. et Chim.* [6], iii., 233, 1896.

¹³ Fermi and Montisano, *Apothekerzeitg.*, ix., 583, 1894.

¹⁴ Moriggia and Ossi, *Atti Accad. Lincei.*, 1875.

¹⁵ Fubini, *Arch. Ital. d. Biol.*, xiv., 436, 1891.

GÉRARD¹ found an emulsin in the *intestinal secretion* of a rabbit which he had fed for several days with salicin, whilst the *pancreas* contained no emulsin.

In *Cephalopoda* BOURQUELOT² was unable to find any emulsin, nor could he confirm the statement of STAEDLER³ that saliva diastase decomposed *salicin*. He concluded rather that an eventual decomposition of the salicin in the saliva was due to micro-organisms. The same explanation applies to the action of saliva diastase on *amygdalin*, as observed by BOUGAREL.⁴

Mode of Occurrence of Emulsin in Plants.—The relation of *amygdalin* and *emulsin* to the tissues of the plants which produce them has frequently been studied. In the first place we must conclude that they are separate, since almonds do not show any liberation of hydrocyanic acid until mixed with water.

THOMÉ⁵ came to the conclusion that emulsin is only present in bitter almonds, but that amygdalin also occurs in sweet almonds. PFEFFER⁶ located the emulsin in the protoplasm of the cells, whilst the amygdalin, in his opinion, was contained in the liquid of the cell. PORTES⁷ discovered *emulsin* in the embryo of the seed, and amygdalin in the cotyledon. JOHANSEN⁸ found *emulsin* in all the vascular tissue, including that of sweet almonds; the embryo itself does not contain amygdalin, but *only* emulsin.

Subsequently GUIGNARD⁹ made comprehensive investigations on the occurrence of emulsin.

With the aid of a colour reaction—viz., the orange-red colour given with Millon's reagent—he was able to identify emulsin micro-chemically. Thus, he obtained this reaction with the leaves of *Laurocerasus*, but not with the otherwise very similar leaves of *Cerasus lusitanicus*.

He found the emulsin to be located in anatomically well-defined places in the leaves and seeds; in fact, in *distinct groups of cells*, which he was able to isolate, and which yielded the active ferment.

¹ Gérard, *Journ. d. Pharm. et Chim.* [6], iii., 233, 1896; *C. R. Soc. Biol.*, xlviii., 44, 1896.

² Bourquelot, *Digestion chez les Mollusques*, Thèse. Paris, 47, quoted by Hérissé, *loc. cit.*

³ Staedeler, *Journ. de Ch.*, lxxii., 250, 1857.

⁴ Bougarel, *De l'Amygdaline*, Thèse de Pharm., Paris, 1877, quoted by Hérissé, *loc. cit.*

⁵ Thomé, *Botan. Ztg.*, 240, 1865.

⁶ Pfeffer, *Pflanzenphysiol.*, i., 307.

⁷ Portes, *Journ. d. Pharm. et Chim.*, xxvi., 410, 1877.

⁸ Johansen, *Annal. d. Sciences Naturelles, Botanique* [7], vi., 118, 1887.

⁹ Guignard, *Journal de Botan.*, iv., 3, 19, 1890; *J. d. Pharm. et Chim.* [5], xxi., 233, 1890.

Conditions of the Formation of Emulsin.—In the case of the seeds of *Cerasus avium*, HÉRISSEY¹ has shown that emulsin is produced before amygdalin.

Hérissey (*loc. cit.*, 33) has also investigated the conditions of the formation of emulsin in *Aspergillus niger*. His principal results are that the quantities of emulsin fluctuate, becoming less the nearer the mould approaches the production of fruit; and also that the ferment disappears when an excess of food is given, though it re-appears again when the mould is starved.

It may be mentioned, in passing, that, according to the results of PURIEWITSCH,² living mould-fungi decompose *helicin* in addition to other glucosides, but are then destroyed by the salicylic aldehyde produced; the decomposition of amygdalin, however, when the vital activity of moulds has not been crippled by chloroform, follows a different course, no hydrocyanic acid, ammonia, and benzoic acid being produced.

The decomposition of amygdalin by bacteria has been investigated by FERMI and MONTISANO,³ *inter alios*. They found that this power was possessed by different species, though sugar could never be detected among the decomposition-products. Moreover, the bacteria did not decompose the amygdalin when sugar was at their disposal in the nutrient culture-medium. We have, thus, in these results an example of the fact that enzymes are very frequently only produced when the decomposition of the material is of physiological importance to the organism causing the fermentation.

Preparation and Properties of Emulsin.—Emulsin is not known in the pure condition.

ROBIQUET⁴ obtained his *synaptase* from the expressed juice of almonds by precipitation of the mixed proteids with acetic acid, and subsequent precipitations with lead acetate, and finally with alcohol.

THOMSON and RICHARDSON⁵ extracted the fatty substances with ether and precipitated the residual liquid with alcohol.

ORTLOFF⁶ allowed the fats to become rancid, filtered, precipitated with alcohol, dissolved the precipitate in water, and re-precipitated it. A similar method was tried by BUCKLAND W. BULL.⁷ SCHMIDT⁸ found that solutions of emulsin were not rendered turbid by acetic acid and potassium ferrocyanide, and saw in this a means of separating emulsin from the

¹ Hérissey, *loc. cit.*, 83.

² Puriewitsch, *Ber. d. d. botan. Ges.*, xvi., 368, 1898; *Bull. Soc. Biol.* [10], iv., 686, 1897.

³ Fermi and Montisano, *Apoth.-Ztg.*, 583, 1894.

⁴ Robiquet, *J. de Pharm. et Chim.*, xxiv., 336, 1838.

⁵ Thomson and Richardson, *Ann. d. Pharm.*, xxix., 180, 1839.

⁶ Ortloff, *Arch. d. Pharmac.*, xlviii., 12, 1846. Gives the older literature on bitter almonds and their poisonous properties.

⁷ Bull, *Ann. Chem. Pharm.*, lxix., 145, 1849.

⁸ Schmidt, *Dissert.*, Tübingen, 1871.

proteids present as impurities. He then obtained the ferment in the form of a white powder by precipitation with alcohol.

HÉRISSEY¹ extracted finely-pulverised almonds with chloroform-water, removed proteid impurities by the addition of a little glacial acetic acid, and precipitated the ferment with alcohol.

He thus obtained the *emulsin* in the form of a white powder soluble in water. It gave all the ordinary proteid reactions and was lævorotatory.

Analyses have been published by Orloff, by Bull, and by Schmidt, but do not agree with each other. SCHMIDT found—

C, 48.76.
H, 7.15.

N, 14.16.
S, 1.25.

It thus, doubtless, still contains proteids in these preparations, and also probably an *araban* (?), which, on treatment with sulphuric acid, yields arabinose, in which respect it resembles diastase (*q.v.*). It passes through a porcelain filter, though the enzyme of *Aspergillus* is better in this respect than that of almonds.

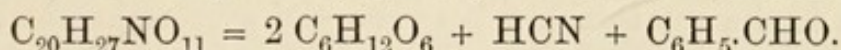
It gives with Millon's reagent a reddish-orange colour, and with *orcin* and hydrochloric acid a violet coloration. The latter reaction is also given by diastase, but not by pepsin and trypsin (GUIGNARD²).

HÉRISSEY was unable to separate the emulsin of the *Aspergillus* from the other enzymes of the mould.

Reactions caused by Emulsin.—Emulsin causes the following decompositions, water being absorbed in the process :—

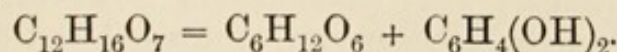
Amygdalin into

2 molecules of glucose, hydrocyanic acid, and benzaldehyde,

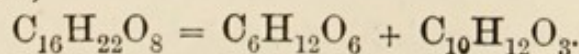


Almond nitrile glucoside, which is formed from amygdalin by the action of yeast enzymes,³ into 1 molecule of glucose, hydrocyanic acid, and benzaldehyde.

The *Arbutin*⁴ of the *Ericaceæ* into 1 molecule of glucose + *hydroquinone*,



The *Coniferin* of *Coniferae* into 1 molecule of glucose and *Coniferyl alcohol*,



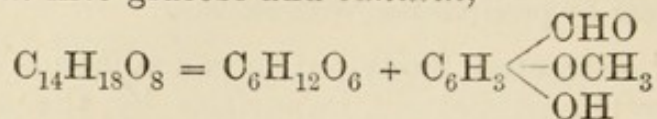
¹ Hérissé, *loc. cit.*, 44, *et seq.*

² Guignard, *Journal de Botan.*, iv., 3, 19, 1890.

³ E. Fischer, *Ber. d. d. chem. Ges.*, xxviii., 1508, 1895.

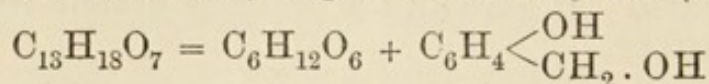
⁴ Kavalier, *J. pract. Ch.*, lviii., 193, 1853.

Glucovanillin into glucose and *vanillin*,



and, in an analogous manner, glucovanillic acid into vanillic acid.

The *Salicin* of varieties of *Populus* into *saligenin* (PIRIA¹),



and, in an analogous manner, its oxidation product *helicin* into *salicylic aldehyde*.

In addition to these, it converts *daphnin* into *daphnetin*, *æsculin* into *æsculetin*, *piceïn* into *piceol*, and, in each case, *glucose*.

According to HÉRISSEY, the emulsin of almonds also decomposes the *gentiopicrin* from *Gentiana lutea*, as well as *syringin* and *phyllirin*, and acts very feebly upon *ononin* and *helleboreïn* (E. FISCHER²). On the other hand, it is without action upon *cyclamin*, *apiin*, and *convallarin*. But, although these are also not attacked by *aspergillus emulsin*, the latter decomposes the glucosides *ononin* and *helleboreïn*, which are only slightly attacked by *almond emulsin*, and also *populin* and *phloridzin*, upon which almond emulsin has no action (HÉRISSEY³).

Both are without influence upon *solanin*, *hesperidin*, *convallamarin*, *convolvulin*, *digitalin* (crystalline), *hederin*, and *quercitrin*. Emulsin does not act upon *monobutylin*, and thus differs from *lipase* (GÉRARD⁴).

Theoretically, it is particularly interesting that *emulsin* decomposes the β -glucosides of fermentable sugars which cannot be attacked by *maltase*, &c., but does not act upon the α -glucosides, which can be decomposed by yeast infusion; and from this E. Fischer has drawn his theoretical deductions given above as to the relationship between the stereo-chemical structure and the action of the enzyme. This observation acquires additional importance from the fact that *emulsin* also decomposes *lactose* into glucose and galactose, and thus acts in an analogous manner to *lactase*. This fact gives us a certain right to assume that there are analogies in the configuration of lactose and that of the β -glucosides. This assumption, however, has a weak spot. For, although Hérisséy has confirmed the decomposition of *lactose* by *almond emulsin*, he has been absolutely unable to effect any decomposition by means of *aspergillus emulsin*.

¹ Piria, *Ann. Chem. Physiol.* [3], xiv., 257, 1845.

² E. Fischer, *Z. physiol. Ch.*, xxvi., 70, 1898.

³ Hérisséy, *loc. cit.*, 57 et seq. Cf. Bourquelot and Hérisséy, *Bull. Soc. Mycol.*, xi., 199, 235, 1895; *id.*, *Journ. d. Pharm. et Chim.* [6], ii., 435, 1895. Hérisséy, *Bull. Soc. Biolog.*, 640, 1896.

⁴ Gérard, *Comptes Rendus*, cxxiv., 370, 1895.

If, then, granting that this observation is correct, we are unwilling to conclude that a *lactase* is also present in *almond emulsin*, it follows that we have here certain differences between the two *emulsins*.

Hérissey claims to have also discovered differences in the velocity of their action, particularly on *arbutin*.

The *optimum* for the action of emulsin varies from 45° to 50° C.,¹ whilst the temperature at which it is destroyed is about 70° C. (HÉRISSEY). In a dry condition it can be heated for an hour at 100° C. without being destroyed (Bull, *loc. cit.*).

It is destroyed by alkalis, but only rendered inactive by hydrochloric acid and other mineral acids²; acetic acid and formic acid have no effect upon it (BOUCHARDAT³).

As regards the action of neutral salts, including those of the heavy metals, only a few—*e.g.*, ammonium carbonate and copper sulphate—retard the action.

In glycerin, amygdalin and emulsin do not act upon one another (SCHMIDT⁴).

The action of other ferments upon emulsin is uncertain; trypsin, however, is without influence. In the *gastric juice* it is at first inactive, since on introducing amygdalin and emulsin into the stomach simultaneously the animal dies of hydrocyanic acid poisoning (CLAUDE BERNARD⁵).

Chloroform, *ether*, *thymol*, &c., have no action upon it, nor has hydrocyanic acid.

From the fact that it is also unaffected by *chloral*, BOUGAREL⁶ attempts to draw the conclusion that emulsin is not a proteid, since chloral forms solid compounds with proteids.

According to HÉRISSEY (*loc. cit.*, 81), *emulsin* is precipitated by *tannin*, but the precipitate still remains active.

Gaultherase.—An enzyme which has a specific action upon the *glucoside* of the *methyl ester of salicylic acid* has been found in several plants in which that glucoside occurs.

It was first discovered by PROCTER.⁷

It was then found by SCHNEEGANS⁸ in *species of Betula*, and named *betulase*. BOURQUELOT⁹ found it in *Betula*, *Spiræa*

¹ Tamman, *Z. physiol. Ch.*, xvi., 271, 1892.

² Jacobson, *Z. physiol. Ch.*, xvi., 1892.

³ Bouchardat, *Comptes Rendus*, xx., 111., 1845.

⁴ Schmidt, *Diss. Tüb.*, 1871.

⁵ Cl. Bernard, *Lec. pathol. Expér.*, Paris, 75, 1890.

⁶ Quoted by Hérissey, *loc. cit.*

⁷ Procter, *Amer. Journ. Pharm.*, xv., 241; quoted by Bourquelot, *loc. cit.*

⁸ Schneegans, *Journ. d. Pharm. von Els.-Lothr.*, 17, 1896.

⁹ Bourquelot, *Journ. d. Pharm. Chim.*, 1896 (June), Reprint.

ulmaria, and *S. filipendula*, in *Monotropa hypopitys*, &c., and also in *Gaultheria procumbens*. Since the glucoside was first discovered in *Gaultheria* and named *gaultherin* (SCHNEEGANS and GEROCK¹), Bourquelot gave the name *gaultherase* to the corresponding enzyme. It does not act upon *salicin* and *amygdalin*, and in this respect differs from *emulsin*. BEYERINCK² has confirmed these statements, and described a method of obtaining an active preparation of gaultherase by extraction with water and precipitation with alcohol.

Myrosin.—The characteristic pungent-smelling and tasting principle of many *Cruciferae*, notably of *black mustard seed*, has from an early period attracted the attention of the investigator. As we gather from an interesting historical survey by SPATZIER,³ the active principle, the *mustard oil*, was probably known to LEFÉBRE in the year 1660, and was certainly known to BOERHAVE in 1775. THIBIERGE⁴ was the first to separate the mustard oil from the juice by distillation and to discover the presence of sulphur in it, and THOMSON⁵ continued and extended his researches. An important advance towards the discovery of the process was made by BOUTRON-CHARLARD and ROUBIQUET⁶ and by FAURE,⁷ who made the discovery that the *mustard oil* did not exist ready-formed in the seeds, but was first produced on contact with water. The peculiar active principle in this decomposition was subsequently indicated by BOUTRON-CHARLARD and FRÉMY.⁸

The actual discovery, however, of *myrosin* must be attributed to BUSSY.⁹ He was the first to distinguish in the emulsion of mustard-seed the active principle, the ferment *myrosin*, from the *glucoside* undergoing decomposition, *potassium myronate*. He pointed out its relationship to the similar *emulsin*, but did not fail to recognise the differences in their specific action.

Potassium myronate was then further examined by LUDWIG and LANGE,¹⁰ and a formula, which, of course, was not quite correct, assigned to it. For the full explanation of the chemistry

¹ Schneegans and Gerock, *Archiv. d. Pharmacie*, vol. cclii., 437, 1894.

² Beyerinck, *C. f. Bakt.* [II.], v., 425, 1899.

³ Spatzier, *Pringsheim's Jahrb. f. wissenschaft. Botanik.*, xxv., 93, 1893.

⁴ Thibierge, *Journal de Pharmacie*, v., 439, 1819.

⁵ Thomson, *ibid.*, 448.

⁶ Boutron-Charlard and Robiquet, *Journ. d. Pharm.*, xvii., 279.

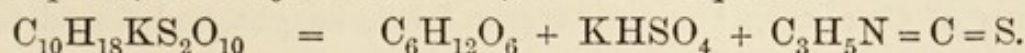
⁷ Fauré, *ibid.*, 299.

⁸ Boutron-Charlard and Frémy, *Lieb. Ann.* xxxiv., 230, 1840.

⁹ Bussy, *Liebig's Ann. d. Chem. u. Pharm.*, xxxiv., 223, 1840.

¹⁰ Ludwig and Lange, *Zeitsch. f. Pharm.*, iii., 430, 577, quoted by Will and Körner (see Note 1, next page).

of the process we are indebted to WILL and KÖRNER.¹ According to them, the glucoside, potassium myronate, is decomposed under the influence of *myrosin* into *grape sugar*, *potassium bisulphate*, and *allyl mustard oil*, as in the equation



Thus it does not follow from this equation that the elements of water enter into the process; and we should not, according to this, be justified in describing the process as simply a hydrolytic one. Nevertheless, *myrosin* is, as a rule, grouped with the hydrolytic ferments, inasmuch as the process takes place exclusively in an aqueous solution, and doubtless involves an intermediate absorption of the elements of water.

The ferment, which appears to be the same wherever it is found in *Cruciferae* (SMITH²), has not been isolated in even an approximately pure condition,³ but shows, without exception, the ordinary reactions of ferments. The colour reactions, by the aid of which attempts have been made to detect it in plants (*vide infra*), can scarcely be attributed to the ferment itself, but are due to the constant presence of other substances, especially proteids. The conditions of its activity are also completely analogous to those of other ferments. According to SMITH,⁴ however, it is active even at 0° C.

Occurrence of the Ferment.—As soon as the presence of mustard oil in *black* mustard seed (*Sinapis nigra*) had been discovered, similar oils containing sulphur were looked for in other plants, without any attention being paid as to the mode of their formation.

Thus, *mustard oil* was discovered in horse-radish oil by HUBATKA,⁵ and in the roots of *alliaria* by WERTHEIM,⁶ and a product differing somewhat from allyl mustard oil in *Cochlearia officinalis* by SIMON,⁷ on stirring the old plant which no longer had any odour with fresh mustard flour containing myrosin. It was afterwards identified by A. W. HOFMANN⁸ as a secondary butyl mustard oil. Eventually, mustard oils were discovered by PLESS⁹ in the seeds of numerous *Cruciferae*, but were not present in a ready-formed condition. VOLLRATH¹⁰ was able to detect it in other plants, viz., in some species of the *Resedaceae*.

¹ Will and Körner, *Lieb. Ann.*, cxxv., 257, 1863.

² Smith, *Z. f. physiol. Ch.*, xii., 432, 1886.

³ *Vide* Will and Laubenheimer, *Liebig's Ann.*, cxcix, 162, 1879.

⁴ Schmidt, *Ber. d. d. chem. Ges.*, x., 187, 1877.

⁵ Hubatka, *Lieb. Ann.*, xlvii., 157, 1843.

⁶ Wertheim, *Lieb. Ann.*, lii., 52.

⁷ Simon, *Poggend. Ann.*, l., 377, 1840.

⁸ Hofmann, *Ber. d. d. chem. Ges.*, vii., 509, 1874.

⁹ Pless, *Lieb. Ann.*, lviii., 36, 1846.

¹⁰ Vollrath, *Arch. d. Pharm.* (II.), cxlviii., 156, 1871.

Subsequently, however, the simple detection of *mustard oils* was no longer regarded as sufficient, but search was made for the glucoside and for the corresponding ferment. Mention should be made here, in particular, of the systematic investigations of GUIGNARD¹ and SPATZIER,² who sought and found the ferment in the organs of numerous plants.

As regards the *glucosides*, to give them the preference, only two are known—*potassium myronate*, which, in addition to its occurrence in black mustard seed, is also found, *e.g.*, in *Brassica*, *Capsella*, and *Cochlearia*; and the *sinalbin* of *white mustard*, which has been more fully investigated by WILL and LAUBENHEIMER.³ It resembles *potassium myronate*, but has a more complex structure.

The remaining glucosides of the *Cruciferae* and other plants which yield mustard oils and grape sugar on decomposition with myrosin are not yet known; but that we have here to deal with glucosides is shown by the fact that Spatzier invariably found that sugar was split off by the action of the ferment.

All these glucosides then are decomposed by *myrosin*, though it does not attack either α - or β -methylglucoside (E. FISCHER⁴). *Cheiranthus cheiri* (*wallflower*) also contains *myrosin*, but no *glucoside*.

For the detection of the ferment in plants, Spatzier looked for the characteristic odour after the addition of *potassium myronate* to the juice of the plant under examination. When an odour was already there, he expelled the ready-formed oil by gentle heat before applying the test. In this way he succeeded in proving that most *Cruciferae*, both the plants themselves and the seeds contain *myrosin*; it was not present, however, *e.g.*, in *Capsella bursa pastoris*. He also found it in the epidermis of the parts of the plant above ground and in the seeds of some *Resedaceae*, but *only* in the seeds of some *Violaceae* and *Tropaeolaceae*.

BOKORNY⁵ found ferments resembling *myrosin* also in *Leguminosae*, *Umbelliferae*, and species of lilies, but glucosides yielding mustard oil only in *Cruciferae*.

So much for the *distribution* of the ferment. Its location and mode of secretion was first studied more closely by GUIGNARD.⁶ He found by micro-chemical reactions that its seat was in special

¹ Guignard, *Journ. de Botan.*, 385, 1890.

² Spatzier, *Pringsheim's Jb.*, xxv., 39, 1893.

³ Will and Laubenhimer, *Lieb. Ann.*, excix., 162, 1879.

⁴ E. Fischer, *Ber. d. d. chem. Ges.*, xxvii., 3483, 1894.

⁵ Bokorny, *Chem. Zeit.*, xxiv., 771, 817, 832, 1900.

⁶ Guignard, *Journal de Botanique*, 385, 1900.

dispersed cells, first observed by HEINRICHER,¹ which contained neither fat nor chlorophyll, but which gave strong albumin reactions. These he named *albumin tubes*. They contained granular masses, which were coagulated by alcohol and gave a more intense coloration than protoplasm with Millon's reagent. He found these cells distributed throughout the whole plant, but the seeds were found to be the richest in them. The glucoside was stored up in other cells. He was able to isolate the ferment with special ease from the wallflower, in the phloemsheath of whose stem it is stored up abundantly.

SPATZIER (*loc. cit.*) continued these researches. With the aid of certain colour-reactions, specially *orcein* and hydrochloric acid, and also by means of the odour formed in the reaction, he obtained the following results:—

The *myrosin tubes* lie either far apart or, as frequently happens, are most intimately connected with the vascular tissue, and can be isolated with this—*e.g.*, in the case of *Cheiranthus cheiri*; the remaining portion of the plant, which has neither vascular tissue nor tubes, contains no *myrosin*. *Capsella*, which has no ferment, also shows no tubes.

In the seeds they lie arranged as in the complete plant—*i.e.*, either diffused or in the procambium. But, whilst the *myrosin* is in a state of solution in the developed plant, it is found in the seeds as solid *granules*. The glucoside and ferment are generally both together in the embryo, though exceptions occur.

The formation of the ferment is independent of the influence of light.

Rhamnase (or Rhamninase) is the name given to an enzyme which occurs exclusively in the seeds of *Rhamnus infectoria* (*Avignon berries*, yellow berries) and was discovered by MARSHALL WARD and DUNLOP.² The fruit contains a glucoside, *xanthorhamnin*, to which the formula $C_{48}H_{66}O_{29}$ has been assigned. On treating the fruit-pulp with an extract of the seeds, the glucoside is decomposed into *rhamnin* (*rhamnetin*) and *glucose*. The ferment has its seat in the raphe of the seeds, the cells of which contain a fatty, glistening, colourless substance. The glucoside, as is invariably the case, is stored up in other cells.

The ferment is destroyed on boiling. CH. and J. TANRET³

¹ Heinricher, *Mittheil. a. d. bot. Inst. Graz*, 1886. Quoted by Spatzier, *loc. cit.*

² Marshall Ward and Dunlop, *Annals of Botany*, i., 1, 1887.

³ Ch. and J. Tanret, *Bull. Soc. Chim. de Paris*. Quoted by *Rev. gén. d. Sciences*, 100, 1900.

have found that the glucoside is decomposed in the process into *rhamninose* and other products which have not yet been further examined. *Rhamninose* is stated to be a *trisaccharide* which can be decomposed into two molecules of *rhamnose* (methylpentose) and one molecule of *galactose*. It would thus be the first instance of the discovery of a "glucoside" which does not include *glucose* among its decomposition-products. This assertion, however, is not in accordance with that of WARD and DUNLOP, who found *glucose*.

The Enzyme which Produces Indigo.—Although until quite recently the decomposition of the glucoside *indican* into *indigo white* and *indiglucin* was generally attributed to the activity of micro-organisms, it appears from the interesting results of v. LOOKEREN-CAMPAGNE¹ and BRÉAUDAT² that an enzyme plays a part in the process. The former succeeded, on the one hand, in obtaining in the case of *Indigofera tinctoria*, and the latter in the case of *Isatis alpina* and some other indigo-producing plants, the typical decomposition in the presence of *chloroform-water*, by which bacteria must have been eliminated; whilst, on the other hand, the decomposition could no longer be obtained after previously heating the leaves or *boiling* the sap. Bréaudat infers the consecutive action of a *hydrolytic ferment* which effects the decomposition into *indigo white* and *indiglucin*, and of an *oxydase* which oxidises the *indigo white* to *indigo blue*.

Other Ferments which Decompose Glucosides.—We have still to refer briefly to some other ferments which decompose glucosides, and which have as yet been very imperfectly examined.

The glucoside of *madder* (*Rubia tinctoria*), *ruberythric acid*, is decomposed by a ferment, *erythrozyme*, simultaneously present, into *alizarin* and *glucose*.³ *Emulsin* has a similar, but much weaker, action; on the other hand, the ferment has no influence upon *amygdalin*.

Other ferments are described by SCHÜTZENBERGER⁴ which decompose *phyllirin* (from *Phillyrea latifolia*) and *populin* and also one which decomposes *tannin* (?). A ferment which is said to only decompose *salicin* was found by KRAUCH⁵ in pumpkins.

¹ v. Lookeren-Campagne, *Landw. Versuchsstat.*, xliii., 401, 1894. Quoted by Koch's *Jb.*, 289, 1894.

² Bréaudat, *Comptes Rendus*, cxxvii., 769, 1898.

³ Schunck, *J. pr. Ch.*, lxiii., 222, 1854.

⁴ Schützenberger, *Die Gährungserscheinungen. Internat. Wiss. Bibl.*, 271, 1876.

⁵ Krauch, *Landwirthsch. Versuchsstat.*, xxiii., 77.

BERG¹ claims to have obtained a "new" glucoside-decomposing enzyme from *Ecballium elaterium*, which, according to him, produces *elaterin* by decomposition from a glucoside, and of course, above all, is designated *elaterase*. Since, however, it also decomposes *amygdalin* as well as starch and cane sugar, we may surely venture to ascribe its specific activity to *emulsin*.

¹ Berg, *Bull. Soc. Chim.* [3], xvii., 85, 1897.

CHAPTER XIX.

OTHER HYDROLYTIC FERMENTS.

FAT-DECOMPOSING FERMENTS.

THE lipolytic ferments, which are also named *steapsins*¹ or *lipases*² possess the capacity of decomposing *neutral fats*.

Fats are esters of *glycerin*, a trivalent alcohol of the formula $\text{CH}_2.\text{OH}.\text{CHOH}.\text{CH}_2.\text{OH}$, with the so-called *fatty acids*, the most important of which are *palmitic acid*, $\text{C}_{15}\text{H}_{31}.\text{COOH}$, *stearic acid*, $\text{C}_{17}\text{H}_{35}.\text{COOH}$, and the unsaturated *oleic acid*, $\text{C}_{17}\text{H}_{33}.\text{COOH}$. The fats themselves are therefore designated *palmitin*, *stearin*, and *olein*.

Under the influence of the fat-decomposing enzymes they are resolved into their constituents *glycerin* and *free acids*. It is thus possible to recognise and measure the activity of the ferments in decomposing fats by detecting the acids by means of their reaction towards litmus and eventually estimating them quantitatively by titration with alkali.

The decomposing action of the *pancreatic juice* upon fats has been known the longest.

The first observation of the pancreas having an influence upon fats was made by EBERLE,³ who noticed an emulsification.

CLAUDE BERNARD⁴ investigated this property more fully. He regarded the emulsification as of primary importance, and therefore termed the active force *ferment emulsif*.

This emulsification, however, is only a *secondary* phenomenon, which can invariably be observed in fats when they are *saponified* to a very slight extent. As soon as a trace of an alkali salt of a fatty acid is present, an emulsion occurs on shaking with water. The primary phenomenon in the action of the pancreas enzyme is the splitting off of free fatty acid, which then forms with the

¹ Biedermann, *Pflüg. A.*, lxxii., 157, 1898.

² Hanriot, *Comptes Rendus*, cxxiii., 753, 1896.

³ Eberle, *Physiologie der Verdauung*, Würzburg, 1834.

⁴ Cl. Bernard, *Physiolog. Expér.*, ii.

sodium carbonate of the intestinal secretion the soap required for an emulsion.

The acid reaction was first noticed by Cl. Bernard. It can be demonstrated in a remarkably striking manner with the aid of litmus in a neutral ethereal solution of *butter*.

BERTHELOT¹ showed that the synthetically-prepared glycerin ester, *monobutylin*, was decomposed into glycerin and butyric acid. The ferment is said to also decompose the esters of other acids—*e.g.*, the *ester of acetic acid* (HERITSCH²).

The quantity of ferment in the pancreas varies, being least six hours after a meal, and greatest in starving animals (GRÜTZNER³).

Attempts to isolate the ferment have not yet passed the initial stage. By extraction with glycerin GRÜTZNER³ has obtained infusions of pancreas which possess the lipolytic function.

The difficulty of isolating the ferment is due to its extraordinary sensitiveness towards acids in particular, but also, as it appears, to *common salt*, &c. It can therefore only be obtained from absolutely *fresh* pancreas.

A quantitative method of estimating approximately the amount of its action has been given by Grützner, *loc. cit.* He counts the number of drops of a solution of the ferment which are required to decompose known quantities of an emulsion of almond oil. Another is based upon the saponification of *monobutylin* and titration of the liberated butyric acid (HANRIOT and CAMUS⁴).

J. H. KASTLE and A. S. LOEVENHART⁵ have recently found that *ethyl butyrate* is much more rapidly hydrolysed than glycerin esters, and is therefore more suitable for the estimation of the activity of the enzyme. In their experiments they macerated the fresh pancreas with coarse sand and extracted the enzyme with water or glycerin. One c.c. of the extract thus obtained from 10, 20, or 50 grammes of the tissue and diluted to 100 c.c. was allowed to act for forty minutes on a mixture of 4 c.c. of water, 0.1 c.c. of toluene (as preservative), and 0.25 c.c. of ethyl butyrate at a temperature of 40° C., after which the liquid was titrated with $\frac{N}{20}$ potassium hydroxide.

¹ Berthelot, quoted by Gamgee, *Phys. Ch. d. Verdauung*; translated into German by Asher and Beyer, 225, 1897.

² Heritsch, *Centralbl. med. Wiss.*, 449, 1875.

³ Grützner, *Pflüg. A.*, xii., 302, 1876.

⁴ Hanriot and Camus, *Comptes Rendus*, cxxiv., 235, 1897.

⁵ J. H. Kastle and A. S. Loevenhart., *Amer. Chem. J.*, xxiv., 491-525, 1900.

Compared with the pancreas, the other tissues of the pig showed the following relative degrees of activity:—Pancreas, 1.0; liver, 2.93; kidney, 0.50; submaxillary gland, 0.36.

Comparative experiments were made with the livers of other animals, the action of the enzyme being continued for fifteen minutes. Per cent. of hydrolysis:—Pig, 8.66; sheep, 4.77; duck, 2.70; ox, 2.20; and chicken, 1.95.

Lipase was found to be almost completely removed from its "solution" by repeated filtration.

It was also found to be more stable than is usually supposed, for extracts of the liver and pancreas could be kept several days without losing their activity. Thus, extracts kept in a refrigerator at 1° C. for several days showed the following per cent. of hydrolysis:—Initial extract, 4.09; after 48 hours, 4.44; after 72 hours, 4.14.

The increase in the activity was attributed to the conversion of zymogen into enzyme.

Ethereal salts were most rapidly hydrolysed by lipase at 40° C. At 60° to 70° C. the enzyme was destroyed.

Most of the common antiseptics were injurious to the enzyme, and particularly sodium fluoride, hydrofluoric acid, and acids in general.

As regards the kinetics of the reaction, it was found that—1. The velocity was not proportional to the active mass of the ethereal salt. 2. The velocity was nearly proportional to the concentration of the enzyme. 3. The reaction was incomplete; but in the case of very concentrated or active extracts of the enzyme, and with very small amounts of ethereal salt, it was nearly, if not quite, complete. 4. The coefficient of velocity was not constant, but decreased with the progress of the reaction.

By means of lipase Kastle and Loevenhart have effected a synthesis of ethyl butyrate from butyric acid and alcohol, from which they conclude that the action of the enzyme is a reversible one. They consider that this has a bearing on the question of the storage and utilisation of reserve fatty-material in plants.

The ferment appears not to be confined to the pancreas.

SCHMIEDEBERG¹ isolated his *histozyme*, which can decompose both fats and *hippuric acid*, from the kidneys, liver, and blood.

HANRIOT² discovered *lipase* in the blood and serum of nearly all the animals examined by him, and also in the liver, where he detected and quantitatively estimated it with the aid of *monobutyrin* (*vide supra*) (HANRIOT and CAMUS³).

According to KNAUTHE,⁴ it is contained in the intestines of fish.

In the intestine of insects, notably the *meal-worm* (*Tenebrio molitor*), a very active lipolytic ferment was found by BIEDERMANN.⁵

Blood lipase is stated not to originate from the pancreas since

¹ Schmiedeberg, *A. f. exper. Path.*, xiv., 379.

² Hanriot, *C. R. Soc. Biol.*, xlviii., 925; *Comptes Rendus*, cxxiii., 753.

³ Hanriot and Camus, *Comptes Rendus*, cxxiii., 831; cxxiv., 235, 1897.

⁴ Knauthe, *Du Bois A.*, 149, 1898.

⁵ Biedermann, *Pflüg. A.*, lxxii., 157, 1898.

it differs from pancreas lipase, particularly as regards the influence of temperature and of alkalinity upon it (HANRIOT¹). *Hæmo-lipase* is said to be increased, for example, in *diabetes mellitus*, but to be diminished, e.g., in *pneumonia*, *carcinoma*, and *icterus* (ACHARD and CLERC²).

A decomposition of fat occurs in the stomach, but this cannot be of a fermentative character (on account of the acid reaction) (OGATA³).

Lastly, it should be mentioned that KLUG⁴ claims to have discovered a fat-decomposing enzyme in the pancreas, which, according to him, splits off carbon dioxide and hydrogen, but no methane. He did not find it, however, in every pancreas.

According to the investigations of CONNSTEIN,⁵ the assimilation of fat by the organism takes place to far the greatest extent after preliminary *decomposition*, and not by mere *emulsification*, as was formerly assumed. In this process ferments may surely play an important part.

Lipolytic Vegetable Ferments.—The occurrence of fats as reserve material in seeds, and their solution in the process of germination was first observed by MULDER,⁶ and more fully investigated by SACHS,⁷ who came to the conclusion that *starch* was first formed from the fat, which, however, was contradicted by FLEURY.⁸

The first statement that a fat-decomposing enzyme is present during the germination of the seeds of different plants is due to MÜNTZ,⁹ who established the fact of the occurrence of fatty acids, and whose results were confirmed by SCHÜTZENBERGER.¹⁰

The saponification of vegetable fats during putrefaction was observed by BOUSSINGAULT¹¹ and PELOUZE.¹²

GREEN¹³ prepared from the germinating seeds of *Ricinus communis* by extraction with glycerin or sodium chloride

¹ Hanriot, *Comptes Rendus*, cxxiv., 778, 1897.

² Achard and Clerc, *Comptes Rendus*, cxxix., 781, 1899.

³ Ogata, *Du Bois Arch.*, 515, 1881.

⁴ Klug, *Pflüg. A.*, lxx., 329.

⁵ Connstein, *Pflüg. A.*, lxx., 473; lxx., 76. See his Summary, *Medicinische Woche*, 1900, No. 15.

⁶ Mulder, *Chemie des Bieres*, German translation by v. Grimm, 222.

⁷ Sachs, *Botan. Ztg.*, 178, 1859; 342, 1862.

⁸ Fleury, *Annal. d. Chim.* [4], iv., 38, 1865.

⁹ Müntz, *Annal. de Chim.* [4], xxii., 472, 1871.

¹⁰ Schützenberger, *Die Gährungserscheinungen*, Internat. Wiss. Bibl., 263, 1876.

¹¹ Boussingault, quoted by Müntz, *loc. cit.*

¹² Pelouze, *Ann. d. Chim. et Phys.* [3], xlv., 319.

¹³ Green, *Proc. Roy. Soc.*, xlviii., 370, 1890.

solution and subsequent dialysis, active enzymic solutions which liberated free fatty acid from castor oil after a short time at 40° C. The ferment was destroyed by heating the solutions to the boiling point. Acids and alkalies rendered it inactive without destroying it.

It is not stored up in the embryo, as Müntz concluded, but only in the endosperm. According to Green it is present in the form of zymogen in quiescent castor seeds. LUMIA¹ discovered *lipase* in the *pumpkin*, in the *castor oil plant*, and in *cocoa-nuts*.

SIGMUND² found it in many other seeds, though to a less extent in the latent than in the germinating condition. He was able to obtain an active preparation from the aqueous extract by precipitation with alcohol.

Fat-decomposing ferments are also found in micro-organisms. Thus, saponification of the fatty-acid esters takes place in all *processes of putrefaction*.

GÉRARD³ has obtained a *lipase* from *Penicillium*. CAMUS⁴ has found one in the same mould, and also to a lesser extent in *Aspergillus niger*⁵; BIFFEN⁶ too, has discovered one in a mould, belonging to the *saprophytes*, which grows on the living cocoa-nut. On triturating the mycelia with kieselguhr, and filtering under pressure, an extract is obtained which decomposes both cocoanut oil and *monobutylin*. The enzyme can be precipitated with alcohol without losing its activity.

There are also other moulds which grow upon nutrient media containing fat—e.g., *Empusa*, *Cordyceps*, *Cyclonium oleaginum* (BRIZI⁷), and *Inzengœa asterosperma* (BORZI⁸).

According to SIGMUND,⁹ there exists a close relationship between the lipases and the enzymes which decompose glucosides, for he claims to have decomposed fats by means of emulsin, and amygdalin by means of lipase. But, inasmuch as he did not use pure ferments, his experiments are not very convincing. Besides GÉRARD (*loc. cit.*) did not observe *any* action of *emulsin* on *monobutylin*.

¹ Lumia, *Staz. Sperim. Agrar. Ital.*, xxxi., 397; *Maly's Jb.*, 724, 1899.

² Sigmund, *Monatsch. f. Chemie*, xi., 272, 1890.

³ Gérard, *Comptes Rendus*, cxxiv., 370, 1897.

⁴ Camus, *C. R. Soc. Biol.*, xlix., 192, 1897.

⁵ *Id.*, *ibid.*, 230.

⁶ Biffen, *Annals of Botany*, xiii., 336, 1899.

⁷ Brizi, quoted by Biffen, *loc. cit.*

⁸ Borzi, *Botan. Centralbl.*, xxiv., 14, 1885.

⁹ Sigmund, *Monatsh. f. Chemie*; *Maly's Jb.*, 596, 1892.

There are also bacteria which decompose fats—*e.g.*, *B. fluorescens non-liquefaciens* (KRUEGER¹).

As regards pathogenic bacteria, SOMMARUGA² asserts that fats are decomposed—*e.g.*, by the bacteria of *cholera* and *typhus*, and also by *B. pyocyaneus*. Indeed, according to Sommaruga, all bacteria which decompose fat are pathogenic.

The Ammoniacal Fermentation of Urea.

When urine is left exposed to the air it becomes alkaline and acquires a pronounced odour. It was long ago recognised—*e.g.*, by BOERHAVE³—that this was due to the production of ammonia.

VAN HELMONT⁴ had already included the odour among the *fermentative processes*—*i.e.*, putrefaction. When, however, the true composition of urea had been discovered by PROUT,⁵ and it had been found that urea was decomposed into ammonia and carbon dioxide on distillation, FOURCROY and VAUQUELIN⁶ accounted for the production of ammonia by the spontaneous decomposition of urea in the urine.

PROUST⁷ was the first to succeed in preventing urine from undergoing this spontaneous decomposition, and in keeping it unchanged for a long period. LIEBIG,⁸ in accordance with his decomposition theory, inferred the existence of a ferment. After that the *mucus*, and in particular that of pathological urines, was frequently made accountable for this decomposition.⁹ That micro-organisms occurred in such alkaline urine was first observed by SHEARMAN.¹⁰ But their significance was first made clear by the researches of MÜLLER,¹¹ and of PASTEUR¹² and his pupil VAN TIEGHEM.¹³ Pasteur was able to preserve boiled urine

¹ Krueger, *C. f. Bakt.*, vii., 467, 1890.

² Sommaruga, *Z. f. Hyg.*, xviii., 441, 1894 (gives the literature).

³ Boerhave, *Elementa Chimiæ*, ii., London, 1732.

⁴ van Helmont, *Opuscul. medic. inedita*, I. de Lithiasi, 27. Quoted by Leube, *loc. cit.*

⁵ Prout, *Annals of Philos.*, xi., 352, 1818.

⁶ Fourcroy and Vauquelin, *Ann. d. Chim.*, xxxi., 48; xxxii., 80, 113.

⁷ Proust, *ibid.*, 2nd Ser., xiv., 257.

⁸ Liebig, *Chem. Briefe*, xv., 6th Ed., 1878.

⁹ See, *inter alios*, H. Fischer, *Berl. klin. Woch.*, 18, 1864; Hankel, in *Schmidt's Jahrb.*, iii., 1.

¹⁰ Shearman, *Schmidt's Jahrb.*, lv., 276.

¹¹ Müller, *J. pract. Ch.*, lxxxii., 452, 1860.

¹² Pasteur, *Comptes Rendus*, l., 849, 1860.

¹³ van Tieghem, *Comptes Rendus*, lii., 210; lviii., 210, 1864.

without change in hermetically-closed flasks, whilst it underwent decomposition on admitting the air. Van Tieghem invariably found micro-organisms in ammoniacal urine, and these he termed *Torulaceæ*. He seldom observed them alone, but usually in company with infusoria. They also caused the decomposition of *hippuric acid*. That the germs penetrated from the exterior was also confirmed by the experiments of CAZENEUVE and LIVON,¹ who tied up the bladders of living animals, then extirpated them, and were thus able to keep the urine unchanged, even when they had rendered it alkaline or had added albumin or sugar to it. When they surrounded the bladder with paraffin, they found that fermentation occurred in the urine, which dialysed through into the space between the bladder and the paraffin, but not when the paraffin had previously been sterilised. On opening the bladder fermentation soon commenced, and *cocci* could be detected.

MEISSNER,² too, found that fermentation did not occur when air was excluded. LEUBE³ observed that fresh normal urine contained no bacteria, and that, on exposing urine in different places, the fermentation occurred after different periods of time, depending on the quantity of germs which had gained admittance from the air.

MIQUEL⁴ found the germs widely distributed in the atmosphere.

Even in cases of fermentation within the bladder, the germs are usually derived from the exterior—*e.g.*, by catheterising—though LEUBE³ considers that an infection by way of excretion from the kidneys is not out of the question.

That the mere presence of cocci, however, was not sufficient to induce fermentation was shown by the results of GUIARD,⁵ who found that bacteria did not cause fermentation in the *healthy* bladder. The bacteria must thus be rapidly destroyed in the healthy bladder, whilst in diseases of the mucous membrane (*cystitis*) they find a suitable culture medium.

MIQUEL⁶ discovered, in addition to the "*Micrococcus urinæ*," a *Bacillus ureæ* which could withstand being heated to 90° C., and also found subse-

¹ Cazeneuve and Livon, *Comptes Rendus*, lxxxv., 571.

² Meissner, quoted by Leube, *loc. cit.*

³ Leube, *Zeitschr. f. klin. Med.*, iii., 233, 1881; and *Virch. Arch.*, c., 540.

⁴ Miquel, *Bull. Soc. Chim.*, xxix., 387, 1878. Thèse.

⁵ Guiard, *Etude sur la transform. ammon. des Urines*, Thèse, Paris, 1883. Quoted by Leube, *loc. cit.*

⁶ Miquel, *Bull. Soc. Chim.*, xxxi., 391; xxxii., 126, 1879.

quently other *Urococci*, *Urobacilli*, and a *Urosarcina*.¹ V. JAKSCH² discovered polymorphic bacteria which developed their greatest activity at about 33° C., and which required for their development a nutrient medium containing phosphorus, sulphur, potassium, and magnesium, in addition to urea or of a number of other organic substances—*e.g.*, succinic acid, grape sugar, &c. They also need free oxygen.

LEUBE³ then fully investigated the conditions of the fermentation of urine in a very careful manner, using pure sterilised urea free from ammonia. He made pure cultivations of the bacteria occurring in ammoniacal urine, and thus isolated eight or ten species, of which four were found to be active, notably a *Micrococcus ureæ* and a *Bacterium ureæ*.

B. proteus proved to be inactive, but *lung sarcinæ* were active. On the other hand, BRODMEIER⁴ found that *B. proteus vulgaris* was very active. A *Urobacillus Schützenbergii* is described by CAMBIER.⁵

The Enzyme of Urea-Fermentation (Urase).—MUSCULUS⁶ found in urine an unorganised ferment which had the same decomposing action on urea as the bacteria mentioned above, and especially in the thick, mucilaginous, ammoniacal urine of cystitis. By precipitation of this mucilaginous urine with alcohol, he obtained the enzyme in a dry condition and was able to keep it for a long time. It was destroyed by acids and by heating to 80° C., but was not affected by phenol. It was perfectly *specific*, acting only upon urea, which it decomposed into ammonia and carbon dioxide.

According to LADUREAU,⁷ it is also active *in vacuo*, under a pressure of three atmospheres, and in the presence of nitrogen, hydrogen, carbon dioxide, &c.

LEA⁸ was only able by precipitation with alcohol to obtain the ferment from the mucilaginous deposit of cystitis urine, but not from the decanted and filtered urine itself. It was indiffusible. In other particulars he confirmed the results of Musculus. PASTEUR and JOUBERT⁹ proved that the enzyme only occurred when the bacteria which decomposed urea were present, and concluded that these micro-organisms produced the ferment.

¹ Miquel, *Annal de Micrograph.*, i., ii., iii., v. Quoted by Flügge, *Die Micro-organ.*, viii., ix., 1896. Quoted by Koch's *Jb.*, 1896, 1897.

² v. Jaksch, *Z. physiol. Ch.*, v., 395.

³ Leube, *Virch. Arch.*, c., 540.

⁴ Brodmeier, *C. f. Bakter.*, xviii., 380, 1895.

⁵ Cambier, *Ann. d. Microg.* Quoted in Koch's *Jahrb.*, 285, 1893.

⁶ Musculus, *C. R.*, lxxviii., 132, 1874. *Pflüg. Arch.*, xii., 214.

⁷ Ladureau, *Comptes Rendus*, xcix., 877, 1884.

⁸ Lea, *Journ. of Physiol.*, vi., 136.

⁹ Pasteur and Joubert, *Comptes Rendus*, lxxxiii., 5, 1876.

According to LEA,¹ it is firmly attached to the *living* cells, since the filtrate from this deposit has *no* action; not until the cells have been destroyed by alcohol is the enzyme liberated and rendered capable of being extracted by water.

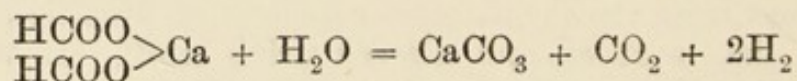
LEUBE,² in like manner, was unable to obtain any soluble ferment by filtration of his pure cultivations. MIQUEL³ subsequently succeeded in effecting the approximate isolation of *urase* from *pure cultivations* of different bacteria in peptone-broth containing ammonium carbonate, by sterilisation by means of a porcelain filter, though this was only possible in the *absence of oxygen*. It underwent decomposition with extraordinary readiness; even at 50° C. it was destroyed in a few hours. *Alcohol*, &c., and *free oxygen* had a very injurious influence. Its optimum temperature was 50° C.

JACOBY⁴ observed the formation of ammonia and the decomposition of hippuric acid and urea in extracts of the liver.

LEONE and SESTINI⁵ have published the results of their investigation on the decomposition of *uric acid* into *ammonium carbonate* under the influence of bacteria. They find that the ammoniacal fermentation of uric acid is brought about by the same micro-organisms as that of urea. GÉRARD⁶ concludes that *urea* is split off as an intermediate product (see *Urea-producing Ferment*).

Decomposition of Calcium Formate.—The decomposition of *Calcium formate* into *Calcium carbonate* and *hydrogen* is a peculiar reaction, which, although effected by the agency of bacteria, yet appears to be a pure and genuine fermentation (POPOFF,⁷ HOPPE-SEYLER⁸).

It takes place as represented in the equation:—



This process causes a positive manifestation of heat, and is thus *exothermic*⁹; it is also to be classified as a true fermentative process from the fact that it is independent of the life of the

¹ Lea, *Journ. of Physiol.*, vi., 136.

² Leube, *Virch. A.*, c., 540.

³ Miquel, *Comptes Rendus*, cxi., 397, 1890.

⁴ *Z. physiol. Ch.*, xxx., 148, 1900.

⁵ Leone and Sestini, *Landwirthsch. Versuchsstat.*, xxxviii., 157, 1891.

⁶ Gérard, *C. R. Soc. Biol.*, 516, 1896.

⁷ Popoff, *Pflüg. A.*, x., 142.

⁸ Hoppe-Seyler, *Pflüg. A.*, xii., 1.

⁹ Cf. Berthelot, *Comptes Rendus*, lix., 901, 1864.

bacteria, and continues after the destruction of the micro-organisms (*e.g.*, by means of ether).

A ferment which affects the hydrolytic decomposition of *taurocholic acid* and *hippuric acid* is excreted by many bacteria. The decomposition also occurs when the bacteria are destroyed by ether (HOPPE-SEYLER¹).

¹ Hoppe-Seyler, *Pflüg. A.*, xii., 1.

CHAPTER XX.

THE LACTIC ACID FERMENTATION.

ALTHOUGH the formation of lactic acid from sugars had long been observed, it was in the earlier days merely classified with the rest of the acid fermentations.

Subsequently it was further recognised that under certain conditions *lactic acid* was produced by spontaneous fermentation from liquids containing sugar—*e.g.*, beetroot juice, fruit juice, milk, &c.¹; great difficulties, however, stood in the way of invariably obtaining the desired result, whilst very frequently unexpected and destructive processes of another kind masked or prevented the formation of lactic acid. Thus, before it was found possible to effect the isolation of the specific micro-organisms, the study of this phenomenon was very tedious. BOUTRON-CHARLARD and FRÉMY,² who were undoubtedly among the first to more closely investigate the lactic acid fermentation, regarded the ferment from Liebig's point of view, and in consequence of the frequent failure of their attempts to obtain pure lactic acid fermentations were forced to the conclusion that there was a *variability* in the ferment, which was able to bring about, according to the conditions, sometimes the lactic acid fermentation and sometimes processes of another nature.

PASTEUR³ then showed that *alcoholic fermentation* and *lactic acid fermentation* were processes which must be absolutely distinguished from one another.

Subsequently the views on the subject became clearer. We now know that the *lactic acid fermentation* is also connected with the presence of *living micro-organisms*, and that it is possible to avoid all those disturbing by-processes by the use of pure cultivations.

The micro-organisms of the lactic acid fermentation were first

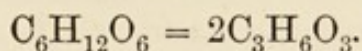
¹ See, *e.g.*, Braconnot, *Ann. Chim. Phys.*, xxxvi., 116; Gay-Lussac and Pelouze, *Ann. Chim. Phys.*, lii., 410, 1883.

² Boutron-Charlard and Frémy, *Ann. Chim. Phys.*, [3] ii., 257, 1841.

³ Pasteur, *Die Alkoholgährg.*, 38.

observed by BLONDEAU,¹ but their significance was not realised. The micro-organisms were then more closely studied, in particular, by PASTEUR.² They were first obtained in a pure cultivation by LISTER.³ The fact that milk could be kept sterile in the absence of air, of which proof had long been sought in vain, was established by ROBERTS⁴ and by MEISSNER.⁵ The biological side of the subject was then thoroughly studied by HUEPPE⁶ with the aid of Koch's method, but to this we shall return presently.

The *chemistry of the reaction* is, in the main, very simple. Lactic acid is formed from the hexoses by simple decomposition, as in the equation



We have here, then, a case analogous to the *myrosin-decomposition*, inasmuch as the absorption of the elements of water plays no perceptible part in the final result, so that we cannot without further proof ascribe this process to the hydrolytic decompositions. According to our view of fermentative action, it would *a priori* be perfectly justifiable to conceive a case in which an unstable molecule merely underwent an internal decomposition with a loss of energy, just as simpler molecules can polymerise without further change with a fixation of energy. Notwithstanding this, however, the lactic acid fermentation is, by fairly general consent, included among the *hydrolytic processes*, an intermediate absorption of water being assumed; and this conception also is completely permissible. Since the question can scarcely be decided experimentally, there is no advantage in discussing it further from a theoretical standpoint, and with this reservation we shall include the lactic acid fermentation among the hydrolytic fermentations.

The resulting lactic acid is almost invariably *α-hydroxypropionic acid*, $\text{CH}_3\text{.CHOH.COOH}$.

HILGER⁷ only once obtained *β-hydroxypropionic acid*, or ethylene lactic acid, $\text{CH}_2\text{.OH.CH}_2\text{.COOH}$, which he was able to identify by oxidising it to malonic acid.

The variety of ethylidene lactic acid formed varies, however,

¹ Blondeau, *Journ. d. Pharm. et Chim.*, xii., 244, 336, 1847.

² Pasteur, *Comptes Rendus*, xlv., 913, 1857; xlvii., 224; xlviii., 337. See also BOUTROUX, *Comptes Rendus*, lxxxvi., 615, 1878.

³ Lister, *Pharmaceut. Journal*, viii., 555, 1877-8.

⁴ Roberts, *Philos. Transact.*, clxiv., 465, 1874.

⁵ Meissner, *Göttinger Chir. Klin.*, quoted by Hueppe, *loc. cit.*

⁶ Hueppe, *Mitth. a. d. kaiserl. Gesundheits.-Amt.*, ii., 309, 1884.

⁷ Hilger, *Ann. Chem. Pharm.*, clx., 336, 1871.

very considerably with the nature of the active agent and of the substratum.

The *fermentation lactic acid* most frequently met with is the racemic *inactive* form, although the *dextrorotatory lactic acid*, the sarcolactic acid of muscle, the zinc salt, of which is *laevorotatory*,¹ is formed frequently enough. Very closely-related species thus produce different acids on the same culture-medium, as is also the case with the *same* species on *different* culture-media.²

We might, indeed, reasonably conclude that in these cases the inactive *racemic* acid is invariably formed first by the primary fermentation, but that certain micro-organisms have the further power, under certain conditions, of consuming the *l*-lactic acid, so that only the *d*-acid is left. It has been definitely proved that they are able to do this in the case of the *ready-formed* racemic lactic acid (FRANKLAND and MACGREGOR³). The production of *l*-acid has been established in one instance by SCHARDINGER.⁴ By making it combine with the *d*-acid, he was able to prepare from it the *racemic* fermentation lactic acid.

We may venture to attribute with certainty this simple decomposition to the action of a ferment which, however, has as yet not been isolated from the cells. But in the lactic acid fermentation, if anywhere, I believe an extension of Buchner's pregnant results to be possible. Apart from the simplicity of the reaction, which it is possible to effect purely chemically in the same way by means of alkalies, there is absolutely no teleological reason to explain why the micro-organisms should expend so much valuable food-material, sugar, to only decompose it in their *metabolism* into a product still endowed with relatively great tension—a decomposition-product, moreover, whose *d*-component they can no longer utilise. We can perhaps construct a physiological picture, as it were, if we form the following conception of the process:—Such micro-organisms as are unable, for some unknown reasons, to assimilate the non-electrolyte, sugar, decompose it by means of an *enzyme* which is produced, as is invariably the case, for this physiological end, and which converts it into racemic lactic acid.

As is also the rule in such cases, this enzyme will provide a large excess of assimilable food-material—in this instance the *electrolyte*, lactic acid. The latter can now be utilised as *food* in such wise that either *both* optical isomerides are further decom-

¹ See, e.g., Nencki and Sieber, *C. f. Bakt.*, ix., 304.

² For the literature, see Flügge, *Micro-organ.*, 233, 1896.

³ Frankland and Macgregor, *Journ. Chem. Soc.*, lxiii., 1028, 1893.

⁴ Schardinger, *Monatsh. f. Chem.*, xi., 545, 1890.

posed indifferently, in which case there remains an excess of *racemic* lactic acid; or that the cell only decomposes the *l*-component when the *d*-lactic acid (or, in certain cases, also the *l*-lactic acid) is left as a decomposition-product which can no longer be attacked. Under favourable conditions of vitality, the micro-organisms appear rather to be capable of further decomposing *both* constituents, so that *racemic* lactic acid invariably is left; if their vitality be weakened, their power of assimilating one or other of the components, according to the circumstances, becomes less, and this conclusion receives support from the experiments of PERÉ¹ on *Coli bacilli*.

But that, in any case, lactic acid is not to be regarded as the *final metabolic product* of the organisms is clear from the invariable presence of *by-products*, even in the case of *pure cultivations*, and to the significance of these in differentiating fermentation from vital metabolism we shall again refer when discussing alcoholic fermentation. In the first place, *carbonic acid* is naturally produced as a purely *vital respiration product* of the organisms, and has no connection with the fermentation as such; the fact of its production has been asserted by HUEPPE² and by ADAMETZ,³ and denied by LEICHMANN,⁴ though with reference, it is true, to other micro-organisms. In addition to this, other products are also formed—*e.g.*, traces of *alcohol* (LEICHMANN⁴), &c.

Subsequently KUPRIANOW⁵ also showed that the consumption of sugar did not run absolutely parallel with the formation of lactic acid, and this we may interpret as indicating that the actual metabolism of the micro-organisms, which depends upon their nature and vital conditions, proceeds simultaneously with the typical *fermentative process*. Moreover, there are means of checking the power of development of the micro-organisms without interfering with the fermentation, *viz.*, *metallic salts* in very slight degrees of concentration (CHASSEVANT and RICHER⁶); this fact admits of the same interpretation. We shall deal more fully with this question as a whole in discussing the very much more important alcoholic fermentation.

Substratum of Lactic Acid Fermentation.—All simple *hexoses* undergo the typical lactic acid fermentation under the influence of the ferment, notably *glucose*, *fructose*, and *galactose*. *Mannite*,

¹ Peré, *Ann. Inst. Pasteur*, vi., 528, 1892; vii., 737, 1893.

² Hueppe, *Mitth. a. d. kaiserl. Gesundh. Amt.*, ii., 309, 1884.

³ Adametz, *C. f. Bakt.* [II.], i., 465, 1895.

⁴ Leichmann, *C. f. Bakt.*, xvi., 826; through *Milchzeitg.*, 33, 1894.

⁵ Kuprianow, *Arch. f. Hyg.*, xix., 282, 1893.

⁶ Chassevant and Richet, *Comptes Rendus*, cxvii., 673, 1893.

&c., however, are also fermented, and likewise *pentoses*, *e.g.*, *rhamnose* (TATE¹).

On the other hand, *cane sugar*, *lactose*, &c., are only fermented to lactic acid, after preliminary decomposition by their special *enzymes*.

Biology of Lactic Acid Fermentation.—The fact that a great number of bacteria cause these fermentations is a further proof that, in the lactic acid fermentation, we have to deal not with a definitely arranged metabolism of distinct species of micro-organisms, but with the production of a ferment, which, like the ferments produced elsewhere by micro-organisms, has a wide distribution.

I cannot undertake to enumerate here all the species of bacteria which produce the lactic acid ferment. There are many of them in *all the groups* of the fission-fungi. Not only *bacilli*, but also *cocci*, *vibrios*, and *sarcinæ* which produce lactic acid are known.² Many pathogenic bacteria—*e.g.*, those of *cholera*, *typhus*, *B. coli*, &c.—must also be included. The production of lactic acid is thus a widely distributed attribute of fission-fungi under definite conditions.

They do not cause fermentation in solutions of pure sugar, although *ammonia* in the form of its salts can serve as their source of nitrogen (TIMPE³).

Peptones appear to be the best culture-medium.

The various organisms which produce lactic acid show considerable differences in regard to the amount produced, as well as in their sensitiveness towards the resulting acid and towards the various by-products, as has been shown by KAYSER⁴ in a comprehensive research. There are both essentially *aërobic* and *anaërobic* forms, whilst others are indifferent to the influence of free oxygen. The cultivations gradually diminish in activity.

Most of them also produce *acetic acid*, which frequently predominates to such an extent that it overwhelms the formation of lactic acid.

Conditions of the Lactic Acid Fermentation.—Since the lactic acid ferment is very closely connected with the life of the cells it is clear that its activity will be destroyed by all agents that destroy the cells. Thus, it is rendered inactive by alkalies, strong acids, and also by all protoplasm poisons, such as the salts of heavy metals, &c. Very minute quantities of these—

¹ Tate, *Journ. Chem. Soc.*, lxiii., 1263, 1893.

² Cf. Flügge, *Micro-organismen*, i., 232, 1896.

³ Timpe, *Arch. f. Hyg.*, xviii., 1, 1893.

⁴ Kayser, *Ann. Inst. Pasteur*, viii., 779, 1894 (gives copious bibliography).

e.g., copper sulphate and mercuric chloride in 0.00005 per cent. solution—are stated by RICHET¹ to have a beneficial influence.

The *optimum* temperature for its activity is 30° to 40° C., but it can resist being heated at 60° C. for a short time (A. MAYER²).

Pepsin has no effect upon it (HIRSCHFELD³).

The micro-organisms which produce lactic acid are peculiarly sensitive to the action of acids, notably *hydrochloric acid*,⁴ and even to lactic acid itself. It is, therefore, necessary to introduce a neutralising agent to obtain a good yield of acid from the fermentation, the most suitable, according to A. MAYER (*loc. cit.*), being calcium carbonate. In milk their sensitiveness is less, which TIMPE⁵ explains by the fact that part of the resulting acid is fixed on the one hand by the *casein*, and on the other hand by the neutral phosphates. Otherwise the fermentation ceases when the amount of acid reaches 0.04 per cent. Gelatin and peptone have a similar fixative action upon the acid.

Is there a Lactic Acid-Forming Enzyme?—It would naturally be of the greatest value in establishing the truth of our theoretical conception of the production of lactic acid, if it were possible to prove the existence of a lactic acid-forming *enzyme*. No such enzyme has as yet been isolated from the organisms which produce lactic acid, but there is another source of the formation of lactic acid, in which an enzyme is *possibly* concerned.

Lactic acid is invariably present in the *organs of animals*, especially in *dying muscle*, and also occasionally in the *urine*.

This formation of lactic acid in the muscle was long ago regarded as a *fermentative* process by DUBOIS-REYMOND,⁶ chiefly because the acetification could be prevented by *heating* the fresh muscle; and in this opinion he was followed by others. NASSE,⁷ in particular, thoroughly investigated the formation of lactic acid, and pointed out numerous analogies between it and enzymic actions.

He came to the conclusion that a hydrolytic decomposition of sugars occurred, and found that it was influenced by certain salts in a perfectly specific manner.

For example, *sulphates* in concentrations up to 9 per cent. had a stimulating influence on the formation of lactic acid, as was also the case with *carbon dioxide*.

¹ Richet, *Comptes Rendus*, cxiv., 1494, 1892.

² A. Mayer, *Maandbl. f. Naturwetensch.*, 1892; *Maly's Jb.*, 598, 1892.

³ Hirschfeld, *Pflüg. Arch.*, xlvii., 510, 1890.

⁴ See, *inter alios*, Cohn, *Z. physiol. Ch.*, xiv., 75, 1890.

⁵ Timpe, *Arch. f. Hygiene*, xviii., 1, 1893.

⁶ Dubois-Reymond, *Sitzb. Berl. Acad.*, *Math.-Physik. Cl.*, 288, 1853.

⁷ Nasse, *Pflüg. A.*, xi., 138.

But, above all, he found that acetification still occurred in an *aqueous extract free from cells*, as had already been shown by KÜHNE.¹

We can thus reasonably form the conception that a ferment is produced in dead muscle and organs, and possibly also *intra vitam*, analogous to the *diastatic* and *oxidising* ferments, which can be obtained from the organs and extracts of organs of animals to the exclusion of vital processes.

Unfortunately, the question of the existence of such a ferment has not yet been experimentally decided. Some experiments, which I commenced a short time ago, have also given results too inconclusive to be advanced with any weight. I have not yet investigated whether there exists in *muscle extract* an active ferment, which, in the absence of putrefactive micro-organisms, converts sugar into lactic acid; I have, however, made a series of experiments to determine whether the so-called *glycolytic ferment* of the blood, with which we shall deal more fully subsequently, does not possibly form lactic acid from sugar. As a matter of fact, I have only been able to obtain very minute quantities of lactic acid from *fresh* horse blood, and also very little from the same blood which had been preserved with toluene and 0.6 per cent. of sodium fluoride, and left for 48 hours in an incubating chamber. On the other hand, I obtained relatively *much* larger quantities when I allowed the blood to stand in contact with a fifth part of its volume of a 2 per cent. solution of grape sugar. I only bring this forward here, however, to show that there is a *possibility* of the existence of a lactic acid-forming enzyme. Possibly I may succeed in finding more weighty reasons in support of my view, in the further results of my experiments, which are still in progress.

¹ Quoted by Neumeister, *Ber. d. d. chem. Ges.*, xxxi., 2963, 1898.

B. THE OXIDISING FERMENTS.

CHAPTER XXI.

THE ALCOHOLIC FERMENTATION.¹

Historical.—The earliest history of the transformation of fermentable sugars by yeast completely coincides with the history of fermentative processes in general. But this process, so eminently important in the practice of the manufacture of alcoholic beverages, was fittingly the type of fermentative processes in general, and when the subject was discussed as a whole it was, in the main, the *vinous* fermentation which, as the chief representative of these processes, was thought of, though *putrid* and *acid fermentations*, as differentiated by Stahl, were still recognised.

There was much speculation and investigation as to the nature of yeast, the nitrogenous character of which had been discovered, and in this connection special mention should be made of FABBRONI, who identified yeast with gluten, of THÉNARD, and of FOURCROY and others. It was found that nearly all animal and vegetable substances were able to produce the "ferments" of alcoholic fermentation.

Then, when the circle of facts requiring investigation had been considerably widened by the discovery of the *unorganised ferments*, LIEBIG attempted to explain fermentative processes as a whole by means of his theory, which is described at length in the "General Part." But when, subsequently, through the researches of PASTEUR,² the great fundamental significance of small vegetable organisms for a great number of "fermentative" processes was brought into the strongest relief, Liebig's *energetic* conception, the theoretical foundation of which showed serious defects, was forced into the background by the *biological* concep-

¹ The name *Fermentation* in this restricted sense is due to FOURCROY, 1787.

² Pasteur, *Die Alkoholgährung*. German translation by Griessmayer, 42. Second Ed., 1878, which gives the earlier history of the subject.

tion based upon the results of these researches. We have shown that it was considered sufficient for the explanation of fermentative processes of this kind to ascribe them exclusively to the vital process of the micro-organisms, relinquishing every dynamic conception and definition, and thus creating in the *organised* ferments a complete antithesis to the *enzymes*. And in this circle of ideas, *alcoholic fermentation* by means of *yeast* again formed the prototype of the processes effected by organised ferments. The active *ferment* in the process was lost in the obscurity of vitalism, and could not, as such, be the subject of discussion. Great energy was shown in the investigation of the *vital conditions* and *morphology* of the *ferment-carriers* on the one hand, and, on the other, in studying the chemistry of the *action of the ferment*.

If, now, we would consider the question of alcoholic fermentation, in which we know by Buchner's experiments that the fermentative action can be separated from the vital process, as a part of the doctrine of *fermentative processes*, it is certain that we must assign as much space to the *chemistry* of the reaction as was ever done before. But the case is different in discussing the *biological* part of our subject. If we start from the standpoint that the yeast cells are only the *parent cells of the ferment properly so-called*, we shall only assign to the biological description of these organisms as much importance for our theme as we have also assigned to the morphological and biological description of the cells which produce *unorganised* enzymes. We shall thus not attempt any detailed outline of the natural history of yeasts, but, in general, merely touch upon them, and only treat them at length in so far as they are bound up with the *fermentative process*, whether with the production or the action of the ferment.

The questions, *e.g.*, of their *botanical position*, of their *food*, of the *forms* and *conditions* of their *growth*, and of their reproduction, are in themselves regarded as belonging to *botany*; only in so far as the *fermentative action* is affected by these influences do these questions come within the scope of our discussion.

But to these two already comprehensive sections a third must yet be added—the question of the *ferment as such*. Our theme thus falls into three divisions—the *nature and production of the ferment*, the *chemistry of its action*, and the *biology of the producers of the ferment*.

The Alcohol-Producing Ferment.—As we have already repeatedly stated, the phenomenon of alcoholic fermentation was, until quite recently, regarded as inseparably bound up with the

vital processes of a small number of *lower fungi*. Although individual investigators came to the conclusion that, notwithstanding this, the alcohol-producing function of these micro-organisms was to be attributed to the activity of *ferments* which differed, of course, from others in the fact that they could not be isolated from the cell, yet an overwhelming majority agreed with Pasteur's view that alcoholic fermentation was exclusively a *purely vital metabolic process* of the fungi. Just as, for example, in the case of the formation of carbohydrates and proteids in higher plants, the production of alcohol was regarded as a vital process of the yeast cells. Such a theory of *destructive* processes within the purely vital process is, indeed, in itself not inconceivable, and even in cases where, beyond doubt, enzymic processes co-operate, as in the transformation of starch in plants, many authorities (*e.g.*, WORTMANN, p. 174), have arrived at the conclusion that, in addition to *the action of diastase*, and possibly *exceeding* it in importance, there is also an action on the part of the living active protoplasm.

So long as it was not found possible to isolate¹ the *enzyme* of alcoholic fermentation from the vital process, no *experimental* solution of this problem, so important for the whole conception of the fermentative process, could be given, and opinion was opposed by opinion.

Only a few results were published which could be cited in support of the view that the *fermentative capacity* and the *life* of yeast were not unconditionally connected, as, for example, the interesting observation of FIECHTER² that *hydrocyanic acid*, whilst destroying the vital process and development of the yeast did not altogether check the *fermentative action*. When relatively larger quantities of yeast were present, hydrocyanic acid suppressed the *fresh production* of ferment as well as the vital process, but did not interfere with the action of that already *formed*. On the other hand, DE BARY'S³ results pointed to the conclusion that it was possible—*e.g.*, in the case of certain *species of mucor* to destroy the fermentative power without destroying life. These facts, however, were never sufficiently convincing to displace the prevailing theory, any more than was the observation of REY-PAILHADE⁴ that an alcoholic extract of yeast (about 20 per cent.) produced carbon dioxide.

¹ Of the many fruitless attempts we may mention that of LÜDERSDORFF (*Poggend. Ann.*, lxvii., 408). He asserted that yeast lost its alcohol-producing capacity on trituration.

² Fiechter, *Wirkg. der Blausäure*, Diss. Basle, 1875.

³ de Bary, *Vorlesg. üb. Bacterien*, Leipzig, 65, 1885.

⁴ Rey-Pailhade, *Comptes Rendus*, cxviii., 201, 1894.

It was thus, then, a scientific discovery of the first rank, when E. BUCHNER proved a few years ago that an enzyme existed which had the power, when isolated from the vital process, of decomposing sugar into alcohol and carbon dioxide.

BUCHNER¹ proceeded in the following manner:—

The yeast was triturated with *quartz sand*, *kieselguhr*, and *water*, and then submitted to a pressure of 400-500 atmospheres between double filter-press cloths. An *expressed liquid* was thus obtained. The residue was again treated in the same way, so that eventually 500 c.c. of pressed extract were produced from 1 kilo. of yeast. It was a faintly opalescent liquid, rich in albuminous matter, and when filtered through a Chamberland filter, or even through filter paper, now contained the *enzyme of yeast*—*zymase*. This pressed extract could be evaporated to dryness at a low temperature (not exceeding 35° C.) without losing its fermentative power. It also retained this capacity when preserved in glycerin.

ALBERT and BUCHNER² subsequently obtained by precipitation with alcohol and ether a *dry preparation* of zymase, which had not lost its alcohol-producing power even after being re-dissolved in glycerin and re-precipitated. The action of the proteolytic ferments, which would otherwise destroy the zymase, was prevented by this means. On the other hand, the liquid which spontaneously exudes from fresh yeast after being mixed with a few drops of ether does *not*, according to ADRIAN,³ possess any alcohol-producing power, though it has an inverting capacity.

Zymase is very unstable. When exposed in solution to the air it loses its activity after a few days, but it can be kept unchanged for a longer time in tightly closed vessels, or in a concentrated solution of cane sugar. Yeast which has been killed by keeping it for a year still contains active ferment (WILL⁴).

BUCHNER⁵ has recently found that yeast is killed by being heated in a current of hydrogen, but that the zymase still remains active, and can subsequently be extracted.

It is destroyed at 40° to 50° C., coagulation occurring simultaneously. With a longer exposure the decomposition takes place at even a lower temperature. On the other hand, it does

¹ E. Buchner, *Ber. d. d. chem. Ges.*, xxx., 117, 1110, 2668; xxxi., 209, 568, 1090, 1531; xxxii., 127, 1897-99.

² Albert and Buchner, *Ber. d. d. chem. Ges.*, xxxiii., 266, 971, 1900.

³ Adrian, *Bull. gén. d. Thérap.*, 156, 1900, quoted in *Chemikerzeitg.*, 76, 1900.

⁴ Will, *Z. ges. Brauw.*, 20, 1896, quoted by Buchner.

⁵ Buchner, *Ber. d. d. chem. Ges.*, xxxiii., 3307.

not appear to be sensitive to the action of dry heat up to 100° C., since yeast thus killed still contains active ferment. This capacity is lost at 150° C. Like a true enzyme it is fairly resistant to the action of chloroform, benzene, and toluene; *sodium arsenite*, which is usually harmless, has sometimes an injurious influence, the cause of which has not yet been explained. It decomposes *hydrogen peroxide*; as in the case of all ferments, this function is prevented by *hydrocyanic acid*, which, however, is also injurious to the action of the ferment. Formalin and hydroxylamine are injurious (WRÓBLEWSKI¹).

It is very sensitive to the action of *proteolytic* ferments, and is, therefore, according to Buchner, rapidly destroyed in pressed extracts which contain such enzymes.

Gentle warmth increases its activity.

Ammonium salts have but little disturbing influence, nor has *sodium azoimide*, but *fluorides* have a marked injurious effect.

The Nature of Zymase.—In spite of all attacks² it has been established by Buchner's researches that alcoholic fermentation is not a vital process of the yeast, but that an active *ferment* is present.

If we describe this ferment, as many wish to,³ as *protoplasmic fragments*, or the like, such a conception is as vague as that of those who would assign to ferments in general *residues of vital force* or similar nebulous attributes.

Protoplasmic fragments, which pass through a Berkenfeldt filter, withstand a dry heat of 100° C., and the activity of which is not crippled by poisons, such as *chloroform* and arsenic, are manifestly no longer *living* protoplasm, and thus cannot exercise any *vital* functions. So long as no proof is brought forward to show that Buchner's expressed liquid contains *living cells* capable of reproduction, these speculative objections to Buchner's views, which are based on *experiments* carefully carried out, are absolutely purposeless. The fact, however, is certain, that the conversion of sugar into alcohol and carbon dioxide is effected by means of a soluble ferment *in the absence* of living cells.

Whether now this enzyme be regarded as more or less complex, or whether a *chemical nature* resembling that of living protoplasm be attributed to it, is so much the less a matter for serious discussion, since the investigations as to the nature of zymase have up to the present yielded very little definite

¹ Wróblewski, *Centralbl. f. Phys.*, xiii., 284, 1899.

² As regards these, see Buchner, *loc. cit.*

³ Abeles, *Ber. d. d. chem. Ges.*, xxxi., 2261, 1898.

information. Buchner is doubtless right in claiming for it an albuminous nature.

The statement that the ferment differs in many particulars from other simple enzymes (NEUMEISTER¹ and WRÓBLEWSKI²) is quite correct. It shows both in its chemical behaviour—*e.g.*, its infinitely greater sensitiveness—and also, in particular, in the conditions of its secretion, considerable differences from other enzymes.

But these differences only show that it is an enzyme of a *special kind*, and one cannot, because it deviates in certain characteristics from other enzymes, therefore refuse to it altogether the character of an enzyme, the definition of which is, in the main, a dynamic one. Zymase completely fulfils this requirement; it is therefore an *enzyme* or, since we cannot regard this term as of primary importance, simply a *ferment*. One need not be surprised that it is the most firmly bound up with the protoplasm, or that it is the most sensitive in the dissolved condition; for we see that ferments which occupy an intermediate position as regards their firmness of attachment, such as *invertase* and *urase*, are undoubtedly more sensitive than those which are readily separated, like *pepsin* and *diastase*. Buchner therefore regards it as closely related to the peculiar *invertase* of *Monilia candida* (*q.v.*), which also cannot be isolated by ordinary methods.

We must therefore conclude that alcoholic fermentation is brought about by an *enzyme* produced by the yeast cells, but that, unlike pepsin, &c., it is not secreted in a free condition in excess, but always diffuses only in trifling quantity from the body of the cell, being rapidly destroyed as soon as it has exerted its specific activity. Possibly there is also a fermentation of sugar which has diffused into the cell in addition to this extra-cellular activity, as may also be the case in the *inversion* effected by *Monilia candida*. However this may be, we have theoretically a true *fermentative process* to deal with here.

The Chemistry of the Reaction.—Until Lavoisier's researches nothing was known of the chemical process of alcoholic fermentation beyond the fact that *alcohol* and *carbon dioxide* were produced by it.

LAVOISIER³ was the first to attempt to follow this process *quantitatively*. He came to the conclusion that *alcohol*, *carbon*

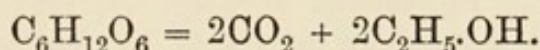
¹ Neumeister, *Ber. d. d. chem. Ges.*, xxxi., 2963, 1898.

² Wróblewski, *C. f. Phys.*, xii., 697, 1898.

³ Lavoisier, *Elém. d. Chim.*, i., 139 (2d Ed.); *Ann. d. Chim.*, ii., 238, 1789; xxxvi., 116.

dioxide, and *acetic acid* were invariably produced. Although this conclusion, as regards a production of acetic acid, at least in the manner believed by Lavoisier, was false, yet an exact determination of the resulting products was impossible on account of the deficiencies of the analytical methods which Lavoisier had at his disposal. Hence it was that Lavoisier represented alcoholic fermentation by an *absolutely false* equation, which has only acquired importance because, in a remarkable manner, the different errors compensated one another in such a way that the sum total of the resulting products almost corresponded to the quantity of sugar used.¹

It was not until the actual composition of *sugar* itself and of *alcohol* had been determined by more accurate analyses, nor until it had been proved that *acetic acid* was not a *normal* product of alcoholic fermentation, that it became possible to replace Lavoisier's erroneous equation by a better one. This important advance was made by GAY-LUSSAC,² who constructed an equation which, translated into our present-day formulæ, reads :—



Gay-Lussac, however, also made a mistake in assigning the equation to *cane sugar* by an arbitrary alteration of figures, whilst, in reality, it belonged to *grape sugar*. This mistake was discovered by DUMAS and BOULLAY,³ who corrected the equation and made the far-reaching observation that cane sugar could not ferment without first taking up a molecule of water.

This equation accurately represents the *main* course of the reaction. Certainly, however, it has been restricted by the fact that alcohol and carbon dioxide are not the only products of the fermentation ; but to this we shall return presently.

If, for the sake of convenience, it were safe to assume that a large proportion of the sugar were decomposed *exactly* into alcohol and carbon dioxide, whilst the by-products were formed independently from other portions of sugar, this equation would be quite justifiable and free from objection.

If, however, we quantitatively follow the fate of the *total quantity of sugar*, this equation does *not* hold good.

Thus, part of the sugar is, as PASTEUR has shown, altogether withdrawn from the fermentative action, in one sense, by the fact that the yeast *consumes* and *assimilates* it—*i.e.*, that it derives from it a part of the necessary carbon for its development.

¹ For further particulars, see Kopp, *Gesch. d. Chemie*, iv., 207 ; and A. Mayer, *Gährungschemie*, 21, Heidelb., 1895.

² Gay-Lussac, *Ann. d. Chimie*, xcv., 311, 1815.

³ Dumas and Boullay, *Ann. Chim. Phys.*, xxxvii., 45, 1828.

Another portion is possibly converted into by-products in a process distinct from the fermentation proper, so that, on this assumption, only a part of the sugar undergoes the true alcoholic fermentation as represented by the above equation.

There are many facts which support the view that the formation of by-products is a process which occurs *simultaneously* with the true fermentation, although the point cannot be decided with certainty.

Thus, for example, the quantity of by-products formed, even in normal alcoholic fermentations, varies considerably, and is influenced by external conditions. *Biological* factors, in particular, especially such as have an influence on the *vital* energy of the yeast cells, play a part in the process, as we shall see below. Taking into consideration the constancy of the chemical nature of other fermentative processes, it is far more probable that these external factors influence the *vital process* of the *yeast cells* than the *fermentative process*. We might thus assume that the formation of these by-products belongs to the domain of the metabolism of the organisms themselves, and that they thus represent typical excretory products, which would naturally show somewhat variable numerical ratios, according to the biological conditions. They would thus be as little "fermentation-products"—*alcoholic fermentation-products* in the theoretical signification—as the products of the *undoubtedly* purely biological transformations which the yeast organism effects from the sugar utilised by it for its *nourishment*; from which it constructs the substances of its cells—cellulose, fats, *proteids*, &c., which, under no circumstances, can we recognise as *fermentation-products*. It is also, again, apparent here how extremely important, even *practically*, is the sharp differentiation between the fermentative *function* and the *biochemical* transformations within the *protoplasm* of the organism which effects the fermentation; and we shall not be surprised that Pasteur did not succeed in also including *these* decompositions in a simple *equation of the process of fermentation*, and that A. MAYER¹ also left the question undecided. In practice it would naturally be the most simple plan, if it were possible, to *prove* that the characteristic fermentative process was confined to a simple decomposition of sugar into alcohol and carbon dioxide, whilst *all* other processes, which, in comparison, quantitatively fall far behind it, were to be attributed to the *metabolic processes* of the yeast. We should then have the simplest conceivable scheme: on the one hand, the typical *fermentative process* acting upon a given part of the

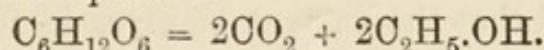
¹ Mayer, *loc. cit.*, 26.

sugar and always following a regular course; and, on the other hand, the *vital process* of the ferment-carriers, depending on the biological conditions, requiring, according to these, a greater or smaller proportion of the sugar for *nourishment*, and leading to secondary decompositions.

From the vital process—*i.e.*, the *respiration* of the micro-organisms—there also results an *increased formation* of carbon dioxide, as compared with the quantity required by the fermentation equation—a fact which Pasteur was long ago able to establish.

On the other hand, however, it is not at present possible to prove that at least the regular production of the two most important by-products, *succinic acid* and *glycerin*, which only varies within slight limits, is to be attributed to another ferment, or rather to two other *ferments*, which also effect *these* specific decompositions—an idea which has been conceived, *e.g.*, by DUCLAUX.¹

This question cannot yet be definitely decided. In any case, however, we must, from our consideration, come to the conclusion that, *under no circumstances*, are the *synthetical* transformations which the sugar undergoes within the yeast cell to be attributed to the *fermentative process*, and that our notions on the nature of this process, regarded from a purely theoretical point of view, will inevitably gain clearness if we regard the *main process* as a simple one which exactly follows the course represented by the equation



The question then arises—Under what category of fermentative processes may we place this process? Thus, at first sight, it may be assigned either to the *hydrolytic* or to the *oxidising* decompositions. The absorption of the elements of water takes no part in the process, if we take into account only the final condition, although it may well be assumed that an intermediate absorption and liberation of water co-operates, since the process occurs exclusively in aqueous solution. For the purpose of classification, the process may be defined as one of *oxidation*, in which, of course, no free oxygen is taken up, but in which part of the molecule is oxidised to the highest stages of oxidation at the expense of the other parts. In this process the intermediate moving of the oxygen results in its *accumulation* in one part of the molecule, which, in consequence, then undergoes decomposition (BAEYER²).

¹ Duclaux, *Ann. Inst. Pasteur*, xi., 348, 1897.

² Baeyer, *Ber. d. d. chem. Ges.*, iii., 73, 1870.

We can the more readily, with all reserve, classify this process as one of oxidation *sui generis*, inasmuch as it is a *combustion phenomenon*—i.e., is accompanied by a *liberation* of energy in the form of heat. It is thus *exothermic*, and in this respect satisfies the requirements which we expect in a fermentative process.

We can therefore, though not without a mental reservation, assign it to the *oxidising* fermentative processes.

The By-Products.—Whilst, as we have mentioned, the products regularly formed to a preponderating extent in alcoholic fermentation are alcohol and carbonic acid, there are also formed as constant products in every normal production other substances, and, above all, *glycerin* and *succinic acid*, which are also met with in fermentations with *pure cultivations of yeast*, so that their presence is not due to foreign micro-organisms.¹

PASTEUR² detected these substances in 1858, and, at the same time, succeeded in preparing them from the mixed fermentation-products in a pure condition.

His method consisted essentially of filtering the liquid from the yeast, freeing it by long-continued evaporation from alcohol and carbon dioxide, and extracting the residue with a mixture of alcohol and ether, in which both glycerin and succinic acid are soluble; the succinic acid was converted into the calcium salt, the glycerin removed by a fresh extraction with alcohol-ether, and the succinic acid obtained in the form of its crystalline calcium salt. This method was subsequently modified—e.g., by FITZ and CLAUSNITZER³ (for the estimation of the glycerin).

A suitable method of determining succinic acid is described by LABORDE and MOREAU.⁴

Pasteur found that the amount of glycerin formed in normal fermentations varied between 2.5 and 3.6 per cent., and that of the succinic acid between 0.4 and 0.7 per cent. of the fermented sugar.

Pasteur then attempted to also include in a comprehensive equation the part which these constant by-products played in the process. We have already expressed the opinion that it would be much more suitable for the prosecution of our theoretical considerations to separate this process from the characteristic fermentative process, and merely to assign it either to a fermentation proceeding on parallel lines or to the *metabolism* of the *ferment-carriers*.

Although the numerical variations in the yield of glycerin and succinic acid are but trifling, they are yet capable of detec-

¹ For the literature, see Flügge, *Micro-organismen*, 226, 1896.

² Pasteur, *Die Alcoholfährung*, 9 (*vide infra*).

³ Cf., *inter alios*, Thylmann and Hilger, *Arch. f. Hyg.*, viii., 451.

⁴ Laborde and Moreau, *Ann. Inst. Past.*, 657, 1899.

tion; they depend, above all, in great measure upon factors which have a special influence upon the quality of the yeast.¹ Pasteur himself had clearly shown that the slower the course of the fermentation, the greater the quantity of by-products formed. A. MAYER² further asserted that a *larger amount* was also produced in neutral fermenting liquids which were less "adapted" to the yeast than in slightly acid liquids. This is also supported by the results obtained by EFFRONT³ and BREFELD,⁴ who found that they were produced to a special degree when only weak alcoholic fermentation was proceeding—*e.g.*, in the last stages of yeast fermentation—or in *mucor* fermentations. On the other hand, according to EFFRONT,⁵ yeast which has been "acclimatised" to sodium fluoride forms less glycerin.

All these facts might, indeed, be interpreted as signifying that when the fermentative *function* of the yeast falls relatively behind, whilst the vital transformation of energy, the *metabolism*, has remained absolutely the same or has been injuriously affected to a smaller extent, the fermentative *main process* is apparently more injured than the other decomposition-processes which lead to the formation of by-products.

A weighty argument in support of this view is the example given that moulds of less *fermentative* capacity, such as certain *species of mucor*, form relatively more by-products. But particularly interesting is the discovery of v. UDRANSKY⁶ that *glycerin* is produced without the liberation of carbon dioxide after the *death* of the yeast, and under conditions resembling those in which all alcoholic fermentation is out of the question, notably in culture-media containing no sugar.

MACH and PORTELE⁷ state that aerated yeast produces more glycerin than that deprived of oxygen, although the *fermentative activity* of the yeast is weaker when oxygen is introduced in abundance. Moreover, according to RAU,⁸ the amount of succinic acid formed is relatively independent of the production of glycerin. At a lower temperature (which involves the *weakening of the metabolism*) less glycerin is produced, but more is formed when the yeast has an excess of nourishment; the production

¹ Cf., *inter alios*, Thylmann and Hilger, *Arch. f. Hyg.*, viii., 451.

² A. Mayer, *loc. cit.*, 28.

³ Effront, *Comptes Rendus*, cxix., 92, 1894.

⁴ Brefeld, *Landwirtsch. Jahrb.*, 281, 1876.

⁵ Effront, *Comptes Rendus*, cxix., 169, 1894.

⁶ v. Udransky, *Z. f. physiol. Ch.*, xiii., 539.

⁷ Mach and Portele, *Landwirtsch. Versuchsstat.*, xli., 1892.

⁸ Rau, *Arch. f. Hyg.*, xiv., 225, 1792.

of succinic acid, however, is not influenced to any extent by these conditions.

Whether *zymase* produces glycerin and succinic acid is not stated in the publications which have yet appeared. It is also difficult to prove, taking into account the usually trifling extent of *zymase* fermentation. A definite negative result would naturally be of the greatest importance for this question.

In addition to glycerin and succinic acid, a very minute quantity (about 0.05 per cent.) of acetic acid is also invariably formed in normal processes of alcoholic fermentation (DUCLAUX¹). Its quantity is so small that its formation can hardly be regarded as a primary product of the typical fermentative process, and, moreover, it shows a significant increase as soon as the biological qualities of the yeast are influenced by pathological processes. Besides, it is only by observing quite exceptional precautions that it is possible to avoid the formation of *more* acetic acid as a secondary product from the alcohol, so that in practice even normally fermented beverages—*e.g.*, *wines*—contain more than 0.1 per cent. of acetic acid.²

As regards other substances in the fermentation-products, Pasteur himself found a very trifling residue of nitrogenous substance, which he did not examine. CLAUDON and MORIN³ also discovered, though, of course, not in fermentations of undoubted purity, small quantities of *amyl alcohol*, and another substance which they regarded as *isobutylene glycol*, $(\text{CH}_3)_2\text{C}(\text{OH})\cdot\text{CH}_2\text{OH}$, besides traces of other substances.

Moreover, MAYER rightly observes that one must in any case conclude that each specific yeast must produce, although often to a very trifling extent, special compounds imparting the odour and flavour, to which particular beverages owe their special value. We may thus, in the present condition of affairs, venture to regard all these by-processes as independent of the typical fermentative process, and to be attributed to the vital process of the yeast.

Such substances are, *e.g.*, traces of *aldehyde* (ROESER⁴), particularly when the air has access, and *acetal*; then the higher alcohols (*fusel oils*) in the mixed products of spirit fermentation (LINDET⁵), *furfural*, and the substances to which wines owe their *bouquet*, consisting of acid esters and ethers.

¹ Duclaux, quoted by A. Mayer, *loc. cit.*, 29.

² A. Mayer, *loc. cit.*, 30; *cf.*, on the other hand, Maumené, *Comptes Rendus*, lvii., 398, 1863.

³ Claudon and Morin, *Comptes Rendus*, civ., 1109, 1887.

⁴ Roeser, *Ann. Inst. Pasteur*, vii., 41, 1893.

⁵ Lindet, *Comptes Rendus*, cvii., 182, 1888; cxii., 102, 1891.

The Substratum of the Process of Alcoholic Fermentation.—The most important discovery from a theoretical point of view in the investigation of what sugars can be fermented into alcohol is the law that in general only sugars with *three, six, and nine* carbon atoms are accessible to the action of the alcohol-producing ferment (E. FISCHER¹). The *triose*² which is produced by the oxidation of glycerin as a mixture of *glyceric aldehyde*, $\text{CH}_2\text{OH}.\text{CHOH}.\text{CHO}$, and *dioxyacetone*, $\text{CH}_2.\text{OH}.\text{CO}.\text{CH}_2.\text{OH}$, and the various *nonoses* are only of synthetical occurrence, and have no special practical significance. On the other hand, it is noteworthy that the *pentoses* (*arabinose, xylose, rhamnose, &c.*), which are so widely distributed in nature, do not undergo fermentation, but only the *hexoses* of the formula $\text{C}_6\text{H}_{12}\text{O}_6$. Here, too, however, we find the peculiar dependence of fermentative processes on the stereo-chemical configuration. Of all the aldehydic sugars the only fermentable ones are *d-glucose* (grape sugar), *d-mannose*, and *d-galactose*, whilst, *e.g.*, *gulose, talose, and idose* are no more fermentable than the *l-forms* of the sugars mentioned above. A special position is occupied by *d-galactose* in this respect. It is *fermentable*, but only by yeasts which are *acclimatised* to it, which, in the case of certain yeasts, can be done with more or less ease. This acclimatisation can also be again withdrawn, notably when there is energetic development of the yeast, or when it is fed with *peptones*. DIENERT³ has studied these questions closely. *S. apiculatus* is stated to have no fermentative action upon galactose (VOIT⁴). Of the *ketoses* only *d-fructose* is known with certainty to be capable of fermentation. The individuality of LOBRY DE BRUYN'S⁵ ψ -*fructose*, which is said to be equally fermentable, has not yet been established beyond doubt. On the other hand, *sorbose* and *tagatose* are not fermentable.

To this extent it can be determined how far sugars are *directly* accessible to the alcohol-producing ferment of the yeast. Yeast, however, is also capable of converting higher carbohy-

¹ Fischer, *Ber. d. d. chem. Ges.*, xxiii., 2137, 1890.

² Great doubt has, moreover, been thrown on the fermentability of glycerose by EMMERLING (*Ber. d. d. chem. Ges.*, xxxii., 342, 1899), and on good grounds. He found that neither glyceric aldehyde nor dioxyacetone, nor *fresh* glycerose, was fermentable, but that fermentation only took place *after heating*, so that one may rather come to the conclusion that there is here a polymerisation into a fermentable hexose.

³ Dienert, see, *e.g.*, *Ann. Inst. Past.*, xiv., 139, 1900; *Chem. Centralbl.*, i., 1033, 1900.

⁴ Voit, *Z. f. Biol.*, xxix., 149, 1892.

⁵ Lobry de Bruyn, *Recueil des Trav. Chim. des Pays-Bas.*, 1897-98.

drates. In the chapter on saccharifying ferments we have shown at length that in addition to the alcohol-forming ferment which is attached to it, the yeast cell also produces a series of enzymes, which first transform the complex carbohydrates into *hexoses*, and thus render them accessible to the action of the typical ferment. We have seen that yeast can decompose *starch* by means of *diastase*, *cane sugar* by means of *invertase*, *maltose* by means of *maltase*, and *trehalose*, *melibiose*, &c., by the corresponding enzymes. Most yeasts are unable to ferment lactose, but there are, on the other hand, special yeasts which produce an enzyme, *lactase*, which decomposes lactose, and then ferment the decomposition-products, *d-glucose* and *d-galactose*. It is remarkable that in these lactose yeasts *maltase* is absent, so that they cannot attack *maltose*. *Maltase* and *lactase* thus appear to be antagonistic in yeast. Others, too, like *S. marxianus*, &c., contain no *maltase*. Some, e.g., like *S. apiculatus* and *S. octosporus*, contain no *invertase*, so that they are unable to attack cane sugar.

The *polysaccharides* are naturally as little capable of direct fermentation. The fact that the *dextrin* which is formed in the action of diastase upon starch is eventually almost completely fermented (BARFOED¹) is attributed to a slow action of the *maltase* (see p. 196). According to KOCH and HOSÆUS,² *glycogen* does not undergo fermentation, but the reverse is the case with the rarer polysaccharides, *helianthin*, and *synarthrin*, which TANRET³ discovered in the roots of Jerusalem artichokes. Within the limits of fermentability further differences are apparent in the velocity of the fermentation of different *hexoses*. Thus, *d-fructose* is fermented more slowly than *d-glucose*, and, in consequence, *cane sugar* more slowly than *maltose*.⁴ In the latter case (*cane sugar*), too, the amount of *fructose* fermented is less than that of the glucose, as was long ago shown by the fact of the decrease in the lævo-rotation of the invert sugar being too small (DUBRUNFAUT⁵).

Zymase ferments *glucose* and *fructose* at the same rate, and *galactose* more slowly, but does not attack *lactose*, *mannite*, &c. *Cane sugar* is fermented by it after being first inverted by the *invertase* contained in the expressed extract (BUCHNER⁶).

¹ Barfoed, *Journ. pr. Ch.*, N.S., vi., 334, 1872.

² Koch and Hosæus, *C. f. Bakt.*, xvi., 145, 1894.

³ Tanret, *Comptes Rendus*, cxvii., 50, 1893.

⁴ Jodlbauer, *Zeits. d. V. f. Rübenzuckerind.*, 308, 1888, quoted by A. Mayer, *loc. cit.*

⁵ Dubrunfaut, quoted by Quévenne, *J. pract. Ch.*, xiv., 334. (See also Bourquelot, *C. R. Soc. Biol.*, 191, 221, 356, 1885.)

⁶ Buchner, *loc. cit.*

The velocity of the enzymic decomposition of polysaccharides does not stand in any constant relation to the velocity of *alcoholic fermentation*, but, on the contrary, this relation varies greatly in different species of yeast.¹

Physical Conditions of Alcoholic Fermentation.—Alcoholic fermentation proceeds best at about 25° C., but the *optimum* temperature varies with the external conditions.

Even below 53° C. the fermentation ceases; in bottom fermentations sometimes as low as 38° C., though, on the other hand, it still occurs at about 0° C. The *optimum* temperature differs in the case of top- and bottom-fermentation yeasts.² Dry yeast resists enormous variations of temperature, from - 113° C. (BERT³) to about 100° C.

According to DUMAS⁴ the duration of the fermentation is approximately proportional to the amount of sugar present; according to A. BROWN,⁵ however, this only holds good for concentrations of 10 to 12 per cent. In the case of higher and lower degrees of concentration less is fermented. COCHIN⁶ has measured the time occupied by it by continuously determining the quantities of carbon dioxide evolved. He found that the fermentation first began after ten to twenty minutes, which was attributed to the slow diffusion through the membranes of the cell. A. BROWN⁵ then plotted the curve of the evolution of the gas, and found that it differed considerably from the curve of ordinary chemical decompositions. According to Brown (*loc. cit.*) and GAYON and DUBOURG,⁷ *diffusion* plays but a very *slight part*, if any at all, since the sugar is not fermented in proportion to its diffusibility.

We cannot deal with the influence of other physical and chemical agents until we come to the biological side of our subject, since this question is inseparable from the consideration of the *biological* significance of these agents.

The Evolution of Heat in Alcoholic Fermentation.—In the simple enzymic processes we had no need to trouble about a consideration of the thermodynamic relations. In that case we had to deal with hydrolytic decompositions, which were also effected by chemical agents, and in which the thermic conditions

¹ Hiepe, referred to by Duclaux, *Ann. Inst. Past.*, xi., 348, 1897.

² A. Mayer, *loc. cit.*, 149.

³ Bert, *Comptes Rendus*, lxxx., 1579.

⁴ Dumas, *Ann. Chim. Phys.* [5], iii., 57, 1874.

⁵ A. Brown, *Journ. Chem. Soc.*, lxi., 369, 1892.

⁶ Cochin, *Comptes Rendus*, cx., 865, 1890.

⁷ Gayon and Dubourg, *Comptes Rendus*, cx., 865, 1890.

of the different substances were investigated in these simple decompositions.

But here we have to deal with a process *sui generis*; only in the alcoholic fermentation itself can the heat evolved by the conversion of sugar into alcohol and carbonic acid be measured.

Such experiments have frequently been made. DUBRUNFAUT¹ found that the increase of heat in the fermentation of a 12 per cent. solution of sugar was equal to that which would raise the heat of this solution by about 14° C. = 0.134 of the heat which the carbon of the resulting carbon dioxide would yield in a direct combustion. BERTHELOT² estimated the positive manifestation of the heat of fermentation at $\frac{1}{15}$ of that which the same quantity of sugar would produce if burned completely.

FITZ³ was the first to attempt to avoid the mistakes which were made in these early experiments, through no notice being taken of the positive increase due to the *heat of solution of the sugar* and of the *heat* produced by the *mixing of the alcohol*, and, on the other hand, of the negative influence of the energy consumed in the *escape* of the carbonic acid. All these factors were taken into account by him.

He found that an 18 per cent. solution of sugar was raised in temperature 21° C. on fermentation, but that 6° was due to the positive evolution of heat *outside* the typical fermentation process.

Is there an Alcohol-Producing Ferment unconnected with Yeast? —The theoretical view that alcoholic fermentation is also a true fermentative process would naturally be considerably strengthened if we succeeded in discovering an analogous process as an *enzymic action* independent of yeast.

Hardly any serious investigations, like those of Buchner, have been made to discover an *enzyme* of alcoholic fermentation in other environments. And yet there is a *possibility* that such ferments actually do exist. Under certain conditions, for instance, there is a formation of *alcohol* and *carbon dioxide* in living organisms.

In *higher plants*, in particular, the formation of alcohol has been observed when *oxygen* was excluded. This fact was discovered by ROLLO,⁴ and has since been repeatedly confirmed.

At a later period the question of the spontaneous formation

¹ Dubrunfaut, *Comptes Rendus*, xlii., 945, 1856.

² Berthelot, *Comptes Rendus*, lix., 904, 1864.

³ Fitz, *Annal. d. Oenolog.*, ii., 428. Quoted by A. Mayer, *loc. cit.*, 161.

⁴ Rollo, quoted by Pfeffer, *Pflanzenphysiologie*, Leipzig, 363, 1881 (gives the older literature).

of alcohol and carbon dioxide in plants was studied, notably by PASTEUR,¹ MÜNTZ,² TRAUBE,³ and LECHARTIER and BELLAMY.⁴ Alcohol is mainly produced when air is excluded—*e.g.*, in the interior of the trunk (DEVAUX,⁵ BERTHELOT⁶), in the seed (MAZÉ⁷), &c.

Even in plucked *fruits*, alcohol frequently occurs (DUMONT,⁸ DÖBEREINER,⁹ GMELIN¹⁰). Yet we must take into account here the possibility that mould-fungi may have been able, in spite of precautions, to penetrate from the exterior surface (BREFELD¹¹). *Evolution of carbon dioxide (without alcohol)* was observed by CAHOURS¹² in plants when oxygen was excluded, and in fresh fruit; and by BÖHM¹³ in green leaves which were kept under water. EFFRONT,¹⁴ too, claims to have detected *zymase* in *cherries*, but has not looked for alcohol.

On the other hand, many mould-fungi produce alcohol in their cells when oxygen is excluded—*e.g.*, *Aspergillus* (PASTEUR), *Penicillium*, *Botrytis* and species of *Oidium* (BREFELD¹¹). Closely connected with these observations is the fact that yeast cells *ferment themselves* when kept in the absence of oxygen in solutions containing no sugar. In this process alcohol and carbonic acid are formed, in addition to the products of proteolytic ferments (*q.v.*).

The statement of SCHUNCK¹⁵ is also interesting—that, in the fermentation of madder, the decomposition of *ruberythric acid* by *erythrozyme* (*q.v.*), the production of alcohol, succinic acid, and carbon dioxide can be detected. As Schunck himself admits, the experiments, which were put aside for a long time, were not made under conditions ensuring the absolute exclusion of micro-organisms. He intends to repeat them, and we may look forward with interest for his results.

¹ Pasteur, *Comptes Rendus*, lxxv., 1056, 1872.

² Müntz, *Comptes Rendus*, lxxxvi., 46, 1878.

³ Traube, *Ber. d. d. chem. Ges.*, vii., 885, 1874.

⁴ Lechartier and Bellamy, *Comptes Rendus*, lxi., 466, 1869; lxxv., 1204; lxxix., 949, 1906.

⁵ Devaux, *Comptes Rendus*, cxxviii., 1346, 1899.

⁶ Berthelot, *Comptes Rendus*, cxxviii., 1366.

⁷ Mazé, *Comptes Rendus*, cxxviii., 1608, 1899; cxxx., 424, 1900.

⁸ Dumont, *Trommsdorff's Journ. f. Pharm.*, iii., [2], 563, 1819.

⁹ Döbereiner, *Schweigger's Journ. f. Chem.*, liv., 418, 1828.

¹⁰ Gmelin, *Handb. d. theoret. Chemie*, ii., 1103, 1829. Quoted by Döpping and Struve, *loc. cit.*

¹¹ Brefeld, *Landw. Jahrb. (Thiel)*, 827, 1876.

¹² Cahours, *Comptes Rendus*, lviii., 495, 635, 1864.

¹³ Böhm, *Sitzb. Wiener Acad.*, lxvii. [1], 211, 1873.

¹⁴ Effront, *Les Diastases*, 302.

¹⁵ Schunck, *Ber. d. d. chem. Ges.*, xxxi., 309, 1898.

Substances of an alcoholic nature also occur in the bodies of animals, and have been investigated by RÖHMANN.¹

Since there was at least a possibility that the sugar-decomposing *glycolytic* ferment (*q.v.*) in the blood might be an alcohol-forming ferment, as the liberation of carbon dioxide has been observed in the course of its action; I have made several series of experiments. In these I have allowed a solution of sugar to stand in contact with fresh blood, with precautions to prevent putrefaction, and have invariably been able to detect in the distillate a very slight quantity of a substance which yields iodoform, and which is *not acetone*. The control experiment with blood alone, however, yielded a distillate (on continued distillation) not completely free from this substance, and a closer examination was impossible with such very minute quantities.²

¹ Röhmann, *Z. f. physiol. Ch.*, v., 103.

² Unpublished experiments.

CHAPTER XXII.

THE BIOLOGY OF ALCOHOLIC FERMENTATION.

Occurrence of the Alcohol-producing Ferment.—Since alcoholic fermentation has, hitherto at least, only been observed in the closest connection with a series of vegetable organisms, the discussion of this subject falls under the head of the biological side of our problem. The investigations into the ultimate cause of alcoholic fermentation became part of the *history of yeast-organisms*, from the moment when, by the classical researches of Pasteur, the significance of these living vegetable cells was irrefutably demonstrated. Prior to that the theory of GAY-LUSSAC,¹ of which an outline was given in the general part—viz., that *oxygen* was the immediate cause which determined the occurrence of alcoholic fermentation—was the one which in general predominated. And when at length the significance of the living cells was recognised, it required a very long time and a hard struggle to lay the ghost of the opposing theory.

The discovery that yeast consisted of round bodies was made by LEEUWENHOEK,² who, however, did not draw any far-reaching conclusions from the fact; nor had the casual statements of DESMAZIÈRES,² who concluded that yeast possessed an organised structure, any greater importance.

The real scientific discovery of the yeast-fungus was afterwards made almost simultaneously by CAGNIARD-LATOUR³ and SCHWANN,⁴ whose conclusions received further support from the results published shortly after by TURPIN,² KÜTZING,⁵ and BOUCHARDAT.⁶ Schwann then opposed Gay-Lussac's theory on the grounds that even in the presence of free oxygen *no fermenta-*

¹ For further particulars, see A. Mayer, *loc. cit.*, 37-40.

² Quoted by Pasteur, *Die Alkoholgährg.*, *loc. cit.*, 42. (See also Kützing, *loc. cit.*).

³ Cagniard-Latour, *Ann. d. Chim. et Phys.* [2], lxxviii., 206, 1836.

⁴ Schwann, *Poggendorff's Ann.*, xli., 184, 1837.

⁵ Kützing, *J. pr. Ch.*, xi., 385.

⁶ Bouchardat, *Comptes Rendus*, xviii., 1120, 1844.

tion resulted when the air in the vessel had previously been strongly heated; and that fermentation also failed to appear on continually introducing fresh quantities of air which had previously been heated. From this he concluded that although the germs of the vegetable organisms which were required for the production of the fermentation process could be introduced into the fermentable liquid through the agency of ordinary air they were not conveyed by air which had been freed from all life by being exposed to a red-hot temperature.

The prevailing opinion in Germany was strongly opposed to these results. The micro-organisms of yeast were partially ignored and partially held up to ridicule in the most bitter fashion.¹ We have described, in the general part, how Liebig himself absolutely refused to be convinced, even at a later date, of the intimate connection between the living fungi and alcoholic fermentation, and how, regardless of this, he formed and defended his decomposition theory. And thus an extraordinarily stubborn battle raged between the newly-formed biological opinion which was upheld, notably by Pasteur, and that of the older school, whose leader was LIEBIG.² As we now look back upon the whole affair, it is much to be regretted that the supporters of a dynamic fermentative activity, independent of life, placed themselves in such direct opposition to Pasteur and his school by denying the biological *facts*; for thereby they were really the first to assist the purely biological *conception* to a victory, which also strongly attracted into sympathy with it the theoretical *explanation* of the process of fermentation. It would surely not have led to such a fundamental separation of "organised" ferments and enzymes, if the supporters of the dynamic conception had plainly admitted the *fact* that alcoholic fermentation, and other similar processes, really did stand in a very intimate connection with the cells of the yeast; but had instead raised the question of how far this undisputed connection with living organisms brought us nearer to an *explanation* of fermentative processes. As a matter of fact, although the discovery of this connection, apart from its great importance to the *fermentation industries*, has a special significance for the question of the origin and biological position of ferments in general, and for the classification of these special fermentative processes; yet it cannot give us an *explanation* of what a

¹ See the silly parody in *Lieb. Ann.*, xxix., 100.

² See, e.g., Schmidt, *Ann. Chem. u. Pharm.*, lxi., 168, 1847, who pretended to have observed fermentation produced by clear filtered yeast-extract, and regarded the chemical theory as "proved."

fermentative process is. On the other hand, the purely biological conception renounces all dynamic investigation of the fermentative process; it reduces the problem to one of the phenomena of the *vital process* which offers to us so many enigmas, and claims to have given an "explanation" of a new problem, by grouping it with an old equally unsolved problem. Thus, in reality, the fight was not for the same object. Liebig contended for a *theoretical conception*; Pasteur, primarily, for a *biological connection*. It is true that Pasteur also formed a *theory* of the action of yeast, based upon his biological discoveries; but, on the one hand, this was also purely biological, completely separating the *process of alcoholic fermentation*, even in theory, from other fermentative processes; whilst, on the other hand, it could be shown to be false in its special assumptions. But since Liebig also opposed the *facts* on which this biological conception was based, as well as all intimate connection between vital activity and fermentation, it followed that with the refutation of his objections his whole point of view had also to give way in favour of Pasteur's conception based upon victorious facts. And it soon became perfectly clear that in the process of alcoholic fermentation, living cells actually did play the important part which Cagniard-Latour and Schwann had ascribed to them.

Proofs which confirmed and extended Schwann's results increased. HELMHOLTZ¹ found that alcoholic fermentation did not pass through membranes—a fact which supported the conclusion of living non-diffusible cells being the cause; SCHRÖDER and v. DUSCH² showed that mere filtration of the air through thick cotton-wool filters was sufficient to prevent fermentation in liquids. All these experiments, however, were incapable of breaking down the resistance of the opponents of this view. Then PASTEUR³ entered the lists, and his experiments proved, with absolute certainty, that *living germs* were really necessary for the production of fermentation, and that these living germs very rapidly gained admission to liquids as soon as they were

¹ Helmholtz, *J. pr. Ch.*, xxxi., 429, 1884; *cf.*, on the other hand, Döpping and Struve, *J. pr. Ch.*, xli., 41, 255, 1847.

² Schröder and v. Dusch, quoted by Mayer, *loc. cit.*, 55.

³ Pasteur made known the results of his researches in numerous publications. See, *inter alia*, *Comptes Rendus*, l., 304, 849, 1083; li., 348, 675; lii., 1260; *Ann. d. Chim. et. Phys.* [3], lviii., 323; [4], xxv., 145 (against Liebig); also his general survey in *Études sur la bière*; *Études sur le vin*; and *Die Alkoholgährung*, German translation by Griessmayer, Stuttgart, 1871, 2nd Ed., 1878. See also the brilliant critical survey by Dumas, *Ann. de Chim. et Phys.* [5], iii., 57.

exposed to the *air*; but that, on the other hand, fermentable liquids could be kept for a very long time unchanged, provided that the ordinary atmosphere were excluded from them. Moreover, Pasteur was able to collect the germs of those organisms on the filters through which he had passed the air, and to cause fermentation by their means in liquids which had previously been boiled, and were thus free from germs. He also showed that on high mountains, where the air contained very few germs, the liquids could usually be exposed to the air for a time, by opening the flasks, without undergoing fermentation. In addition to this, he strengthened the supporters of the biological view by the fact that he was able to detect an increase in the yeast running parallel with the progress of the fermentative process in every fermentation, and by the discovery that the intensity of the fermentative process also varied with the influence brought to bear on the biological functions of the yeast-fungi by alterations in their conditions of nourishment.

Thus, then, by the researches of Pasteur, which received support from the independent investigations of VAN DEN BROEK¹ and HOFFMANN,² from which conclusions of the same kind were drawn, the necessity for the presence of living yeast-cells was clearly established. As regards van den Broek's experiments, special mention should be made of his proof that fresh, unboiled grape-juice does *not* undergo fermentation if the germs in the air be excluded.

On the other hand, proof was brought forward, also by PASTEUR,³ that there could be no question of a *decomposition* of the yeast in alcoholic fermentation in the sense assumed by Liebig, and in which, following Dobereiner's precedent, it had been believed that a liberation of its nitrogen in the form of soluble ammonium salts might be observed. He was able to demonstrate that *absolutely no* ammonia was split off during the fermentation, but that, on the contrary, ammonium salts could be consumed in the process.

By these sledge-hammer blows the opposition of Liebig's school to the biological facts was severely shaken, and Liebig himself, in his last comprehensive experiments,⁴ was forced, in order to maintain his position, to admit the simultaneous production of living organisms with the fermentation.

¹ van den Broek, *Ann. d. Chem. u. Pharm.*, cxv., 75, 1860. See also Wurtz, *Repert. d. Chem. pure.*, 29, 1861.

² Hoffmann, *Bot. Ztg.*, 49, 1860.

³ Pasteur, *Alkoholgähr.*, 56.

⁴ Liebig in his *Annalen.*, cliii., 1, 1870.

After a few small criticisms against Pasteur, which by themselves have little justification, and are comparatively unimportant, he brought forward as his main argument that, as a matter of fact, the yeast secreted a *lifeless enzyme* (*invertase*), which accomplished part of its function, the decomposition of cane sugar. This fact he urged in support of the view that possibly the entire remaining function of the yeast was to be attributed to a *ferment produced* by it. Although he never clearly formulated this meaning of his objection, yet his whole method of argument pointed to the conclusion that he had in his mind a *radical* separation of the *fermentative process* from the *vital process* of the yeast-cells; that the *production* of the ferment, but not its activity, was inseparably bound up with the vegetation of the yeast-fungi; which, indeed—leaving out of the question his untenable assumption of a *decomposition*—is in complete accordance with the theoretical basis of his mode of view.

But this conclusion, which we now know to be very well-grounded, yet which then found in Berthelot, Traube, and Hoppe-Seyler practically its only supporters, was forced into the background through the impossibility of experimentally proving the existence of such enzymes, and this appeared to indicate with absolute certainty the *inseparable* connection of the two processes. Unfortunately, much too little attention was paid, as we observed above, to the purely theoretical question in this discussion, which, for the most part, was restricted to disputes on matters of experiment. Liebig laid too little emphasis on the theoretical question of how far the vitalistic conception *explained* fermentative processes. He was, moreover, not justified in doing so, since he himself was apparently no longer able to uphold his own explanation—the *decomposition theory*. In short, Liebig's attempt to stem the torrent of the vitalistic conception had no further result than to call special attention to a few small weak places in Pasteur's investigations. The experimental results were against Liebig. Neither the study of *invertase* nor the equally active catalytic force of the yeast in decomposing hydrogen peroxide, which was equally outside the question, could be effectively used in support of the view of a separation of the vital process from the fermentative capacity.¹ Moreover, no notice was taken of the few proofs, mentioned above, of the incomplete parallelism of life and fermentative activity. The victory of the vitalistic *conception* with its irrefutable material facts over the dynamic *theory*, based on false premises, was so complete that even Nägeli in his

¹ A. Mayer, *Landw. Versuchsstat.*, xvi., 277.

physico-molecular—i.e., *dynamic* theory of fermentation—came to a standstill before the taboo of “organised” ferments, when he assumed that the atomic vibrations which led to the disruption of the sugar, could *only* proceed from living cells; and that even the highest authorities on alcoholic fermentation, although they perceived that this vital “explanation did not go deeply enough,”¹ contented themselves with adopting it as an *inductive conclusion*, and thus abandoned all attempt at a dynamic explanation of the fermentative processes caused by the yeast.

We have shown in the “General Part” that with this admission all connection with the “fermentative processes” logically falls to the ground, and that Hansen very justifiably also came to this conclusion. As a consequence of this purely vitalistic view, we arrive at the identification of “organised ferments” with the general metabolism, from which point the problem does not admit of separate treatment.

To shortly summarise our survey:—*Theoretically*, the *fermentative function* can be separated from the vital process as such, and it is E. Buchner’s great triumph to have proved that this theoretical separation can also be supported *by experiment*. But even without this experimental support of our view we could theoretically fall back upon Liebig’s endeavour to define fermentative processes as *dynamic*, even though we should also be forced to regard his *theory* as false, and even though we were temporarily unable to replace it by any really better *theory of the activity of ferments*, whether relating to enzymes or “organised” ferments.

Before passing from these theoretical considerations to the *practical* biology of alcoholic fermentation, we must again point out that it cannot come within the scope of this work to give even a merely approximately complete description of the morphology and physiology of those micro-organisms, which must be regarded as the origin of the ferment for this process. We shall merely give a general sketch of the main features of the *morphology* as also of the classification, and only deal more fully with the *physiology* in so far as it is connected with the fermentative function.

Yeast consists of microscopically small globular cell-structures.

Even a superficial study of the most important species of yeast will reveal certain differences in the shape of the individual

¹ A. Mayer, *Gährungschemie*, 67. This admission, however, appears in the main to be only made out of respect to Liebig—a respect which Mayer always religiously paid to the great *savant*, even when the latter was in error.

cells and in their connection with one another. In *bottom-fermentation* beer-yeast, the cells are usually single, or only grouped in pairs, whilst in *top-fermentation* yeast, chains with numerous branches are formed from the single cells. Lastly, *wine-yeast* has smaller individual cells than beer-yeast.

No generally accepted systematic classification has, however, been based upon these obvious differences.

A fundamental distinction for the botanical classification of yeast-fungi was first obtained by the researches of REESS,¹ and these were afterwards supplemented, above all, by the classical investigations of HANSEN,² which have become of the greatest importance to technical fermentation, teaching us how to rear "pure races" of the micro-organisms of fermentation.

Reess designated all fermentation-organisms by the collective generic name of *Saccharomyces*, first used by Meyen, and this has now been universally adopted.

He concluded that there was only one species of beer-yeast, which he termed *S. cerevisiæ*, and explained the differences, particularly those between *top-yeast* and *bottom-yeast*, as "sportive" varieties, and vegetation forms of the same species which had become constant. In the case of *other* alcoholic fermentations, however—wine, brandy, &c.—other *species* were concerned.

S. cerevisiæ occurs in somewhat oblong cells 8 to 9 μ in length.

Reess also made an essential distinction between the following varieties of wine-yeast.

*S. ellipsoideus*³ is slightly elliptical in form, and is 6 μ in length. It forms chains of buds in abundance. It causes the same fermentation as beer-yeast, without itself losing its characteristic form through several generations, and hence there is reason for regarding it as a distinct species. JACQUEMIN⁴ has prepared a *barley-wine* from barley with the aid of *S. ellipsoideus*. In addition to this, *S. apiculatus* also plays a part in the fermentation of wine. It has an oblong form slightly constricted in two places, and is 6 to 8 μ in length. It is interesting from the fact that it produces neither *invertase* nor *maltase*.⁵ Both species are found in nature growing on grapes. According to MÜLLER-THURGAU,⁶ however, the latter is *injurious* in the fermentation

¹ Reess, *Untersuchg. üb. d. Alcoholfährungpilze*, Leipzig, 1870.

² Hansen, Summary in *Untersuchungen aus der Praxis d. Gährungsindustrie* 1895 (gives the literature).

³ Reess, *Ann. d. Oenolog.*, ii., 145, quoted by A. Mayer, *loc. cit.*

⁴ Jacquemin, *Z. f. Rübenzuckerind.*, xx., 267, 1888.

⁵ Amthor, *Z. f. physiol. Ch.*, xii., 558, 1888.

⁶ Müller-Thurgau, *Jahresber. d. deutsch.-schweizer Versuchsstat. f. Obst, Wein u. Gartenbau*, vii. ; *Chem. Centralbl.*, 916, 1899.

of wine. It does not attack *galactose* (VOIT¹). In the *winter* it can be detected in soil (HANSEN²). Besides these there is a third variety—the long (up to 20 μ) club-shaped *S. Pastorianus*, which plays a special part in the after-fermentation of wines rich in sugar.

All these species, again, form “sports” of very different practical value.

The botanical homogeneity of the genus *Saccharomyces* depends, according to Reess, on their typical *mode of reproduction*, in which under certain conditions the ordinary method of increase by budding is interrupted and replaced by the formation of from two to four spores in the parent cells.

It occurs, in particular, when there is a deficiency of food, when an excess of oxygen is present, and under certain conditions of temperature, and also, *e.g.*, in *pressed yeast* (SCHUMACHER³). Reess termed this *ascospore formation*, and in consequence the species of *Saccharomycetes* are classified botanically with the *Ascomycetes*.

We thus find a series of species of *Saccharomycetes* as bearers of the alcoholic ferment. In addition to those mentioned above we also find *inter alios* *S. octosporus*,⁴ *S. exiguus*, which ferments fructose more rapidly than glucose (GAYON and DUBOURG⁵), *S. Ludwigii*,⁶ and *S. Marxianus*. The first, like *S. apiculatus*, contains no invertase; the last two form no maltase (*q.v.*). *S. fragrans* contains no maltase, and, moreover, cannot attack starch (BEYERINCK⁷). To these must be added the *lactose yeasts*, which produce *lactase*—*e.g.*, *S. tyrocola*, and *S. lactis*.⁸

Besides these, there are also certain *Saccharomycetes* in the botanical sense which do *not* ferment sugar into alcohol—*e.g.*, *S. membranæfaciens* (HANSEN), and the formerly so-called *Mycoderma vini*, the *wine-mould fungus*, which Reess also grouped with the *saccharomycetes*.

The numerous experiments to establish relationships between the *mould-fungi* and *yeast* are interesting. A considerable literature has grown up on this point.⁹

¹ Voit, *Z. f. Biolog.*, xxix., 149.

² Hansen, *Z. f. ges. Brauw.*, 449, 1881.

³ Schumacher, *Wiener Acad. Sitzb., Math. Nat. Cl. (I.)*, lxx., 157, 1874.

⁴ This, however, is regarded by Beyerinck, *C. f. Bakt.*, xvi., 49, 1894, as belonging to another genus, and is termed *Schizosaccharomyces*.

⁵ Gayon and Dubourg, *Comptes Rendus*, cx., 865, 1890.

⁶ Cf. Dienert, *Comptes Rendus*, cxxviii., 569, 1899 (Galactose fermentation).

⁷ Beyerinck, *C. f. Bakt. (II.)*, i., 226, 1895 (gives a survey).

⁸ Duclaux, *Ann. Inst. Past.*, i., 573, 1886. Adametz, *C. f. Bakt.*, v., 116, 1889. Kayser, *Ann. Inst. Past.*, v., 395, 1891.

⁹ Cf. A. Mayer, *loc. cit.*, 97 *et seq.* Jörgensen, *C. f. Bakt. (II.)*, i., 321, 1895.

Now that we have attained to a clear view on the subject, this relationship is thus limited:—

Any true intimate connection between these two groups of fungi, botanically absolutely distinct, does *not* exist; the results, which lent support to the notion that true *bud-producing* yeast-fungi might possibly be converted into true *mycelia-producing* mould-fungi were due to the use of insufficiently *pure cultivations*. HANSEN,¹ however, on the one hand, has found that certain true *varieties of saccharomycetes*, under certain conditions, notably in the presence of an abundant supply of oxygen, form skins and mycelial formations, which can only be recognised as true saccharomycetes through retaining their typical spore-formation.

Again, on the other hand, certain *mould-fungi, varieties of Mucor*, and *Aspergillus oryzae* (JUHLE²) are capable, *in the absence of oxygen*, of assuming forms resembling yeast, and of producing buds. These forms then also cause *alcoholic fermentation*, which differs but little from the typical saccharomycetes fermentation. *Mucor mucedo* and *M. racemosus*, in particular, possess this capacity (FITZ³). They produce relatively more carbon dioxide than beer-yeast; neither succinic acid nor glycerin could be detected with certainty; they contain invertase but no lactase. *Mucor alternans* and *M. circinelloides* can also produce alcoholic fermentation (GAYON and DUBOURG⁴). Certain other varieties of mould-fungi are also able to induce alcoholic fermentation, notably *Monilia candida*, which is interesting on account of its characteristic production of invertase, and also the thrush-fungus, which besides alcohol also yields by-products—*e.g.*, aldehyde (LIROSSIER and ROUX⁵).

The koji-ferment in which the *Aspergillus oryzae* is found also forms alcohol and produces *saki*, the rice-wine of the Japanese (see *Taka-diastrase*). The conversion of *Aspergillus oryzae* into a *Saccharomycetes* has been asserted, notably by JUHLER (*loc. cit.*), who has drawn from this, far-fetched conclusions as to the derivation of the *Saccharomycetes*. His conclusions, however, were opposed by KLÖCKER and SCHIÖNNING.⁶ The theory was also severely shaken by the assertion of KOSAI and YABE⁷ that the koji-ferment contained, in addition to *Aspergillus oryzae*, another *yeast-fungus* to which it owed its typical fermentative function. BÜSGEN⁸ had previously come to the conclusion that, contrary to the views of the older investigators, the alcohol-producing function was not to be attributed to the *Aspergillus oryzae* itself. An analogous instance of the co-operation of mould-fungi and *Saccharomycetes* appears to occur in *Tonkin yeast*, in which

¹ Hansen, quoted by Mayer.

² Juhler, *C. f. Bakt.* (II.), i., 16, 326, 1895.

³ Fitz, *Ber. d. d. chem. Ges.*, vi., 48, 1873; ix., 1352, 1876. See also Brefeld, *Ber. d. d. chem. Ges.*, vii., 282, 1875.

⁴ Gayon and Dubourg, *Comptes Rendus*, cx., 865, 1890.

⁵ Linossier and Roux, *Comptes Rendus*, cx., 868, 1890.

⁶ Klöcker and Schiöning, *C. f. Bakt.* [II.], i., 777, 1895.

⁷ Kosai and Yabe, *C. f. Bakt.* [II.], i., 619, 1895.

⁸ Büsgen, *Ber. d. d. bot. Ges.*, iii., 66, 1895.

CALMETTE¹ found alcohol-producing *Saccharomycetes* resembling *S. Pastorianus*, in addition to *Amylomyces Rouxii*, which formed diastase.

Alcohol-producing *Saccharomycetes* also play a part in *pathological mycology*, since in rare cases they cause serious diseases, as, for instance, is done by *S. hominis* (BUSSE²), *S. subcutaneus tumefaciens* (CURTIN³), and *S. neoformans* and *litogenes* (SANFELICE⁴).

Variations in the Fermentative Action.—The various species of *Saccharomycetes* show absolutely no uniformity as regards their alcohol-producing capacity, and, in consequence, their technical utility. Apart from the fact that some of them—e.g., *S. mycoderma*—cause no fermentation at all, the quantity of alcohol which they can yield is also absolutely different. This partly depends upon the fact that some of them do not contain the enzymes required for the hydrolytic decomposition of the cane sugar or maltose, so that they meet with obstructions even in the substratum undergoing fermentation. On the other hand, as the fermentation proceeds the liquid becomes richer in alcohol, which, at a certain degree of concentration stops *all* action on the part of the yeast, and towards which the different species of *Saccharomycetes* vary in sensibility. Thus, for example, *S. apiculatus* ceases to be active relatively soon in the presence of a slight amount of alcohol, even if, as Müller-Thurgau considers, it is not altogether useless for wine-fermentation. The *mucor*-yeasts are still more sensitive to the presence of alcohol.

In practice, however, it is of the greatest importance that many true *Saccharomycetes* form accidental varieties which spoil the beer by the production of by-products which have an unpleasant taste or cause disturbances. It is HANSEN'S⁵ great triumph to have proved that these injurious organisms did not belong to other species of plants, but were actually *Saccharomycetes*.

He then, by laborious investigations, isolated all these accidental varieties from the serviceable one by means of pure cultivations, and taught breweries how to maintain an unvarying good character in their beer by the use of pure cultivations of races of *Saccharomycetes*, which produced *pure* fermentations, whereas; prior to the possession of these pure cultivations, this was practically left to chance. There now exist a whole

¹ Calmette, *Ann. Inst. Past.*, vi., 604, 1892.

² Busse, *C. f. Bakt.*, xvi., 175, 1894.

³ Curtin, *Ann. Inst. Pasteur*, x., 449, 1896 (gives the literature).

⁴ Sanfelice, *Z. f. Hyg.*, xix., 32, 394, 1896.

⁵ Hansen, *Untersuch. a. d. Praxis d. Gährungsindustrie*, 1895.

series of good *races of yeast* which have been grown in pure cultivations, and which, according to the wish of the consumer, yield beer of different, but invariably constant, good flavour.

Certain varieties of, notably, *S. ellipsoideus* (II.) and *S. pastorianus* (I. and II.) were found to be injurious yeasts.

Attempts based on these principles to also obtain the advantages of pure cultivations of yeast for the *wine-fermentation industry*, which previously, contrary to the practice of breweries, had not employed artificially-added yeast, have been made with good results, notably by WORTMANN,¹ but as yet these have not had such a completely revolutionising influence on wine-manufacture as Hansen's experiments had on brewing.

The occurrence of undesirable by-reactions, which Hansen succeeded in preventing by an ingenious method carefully worked out, can, of course, *sometimes* be obviated spontaneously by means of certain precautions which have been empirically devised. This is true, in particular, in the case of the *distillation-industry*, which, of course, is not so sensitive in the matter of flavour (DELBRÜCK²).

Yet here, too, Hansen's method has already begun to be used—*e.g.*, in the *manufacture of rum* (GREG³).

Relationships between the Life of the Yeast and its Fermentative Activity.—When living yeast-cells are cultivated in a solution of sugar, there occur, according to our views, two processes which are theoretically to be kept separate. On the one hand, we have the vital functions of the fungi, their *life* in the narrower sense; and, on the other, their fermentative function—*alcoholic fermentation*. Whilst the latter process is limited to the *sugar*, and, in fact, to the simple *d-hexoses*, the organism of the yeast naturally also requires other *food materials*. The yeast can supply its *carbon requirements* from the sugar which it is able to ferment—*i.e.*, to consume; but for the construction of the proteids which go to compose its framework *nitrogenous food-substances* must also be introduced.

That a source of nitrogen is essential is, *a priori*, self-evident, but yet the definite proof had to be given to the opponents of the vitalistic theory. This was done by Pasteur, who showed that, in a pure solution of sugar free from nitrogen, there was no increase in the yeast or new formation of cells.

We must then inquire further *what* nitrogenous substances can serve as nutriment to the yeast.

Albuminous substances occupy the first place in this respect. Pasteur proved that an extract of the yeast-cells themselves, which contained substances akin to albumin, formed a sufficient nutrient medium for the fungi,

¹ Wortmann, *Landwirthsch. Jahrbücher*, xxiii., 535, 1894. See also *Koch's Jahresb.*, 168, 1894.

² Delbrück, *Wochensch. f. Brauerei*, 1895.

³ Greg, *Centralbl. f. Bakt.*, xv., 46, 1894 (abstract).

but that, on the other hand, the inorganic constituents in the yeast-extract, although containing the *ash of the yeast*, would not support the life of the yeast.

Yeast, like most other plants, cannot assimilate the *free nitrogen* of the atmosphere.

On the other hand, the yeast cells show their exquisite vegetable metabolism in the fact that, as was found by Pasteur, they are able to make use of *ammonium salts*. As available ammonium salts we have not only the *tartrate*, but also the *nitrate*, *oxalate*, &c.

Further highly important sources of nitrogen are found in the diffusible proteids, such as, in particular, *peptones* and similar substances. And to these must be added the proteids of *egg-yolk* and *syntonin*. On the other hand, more complex albuminous substances—*e.g.*, *albumin* and *casein*—are not suitable for the nourishment of yeast, as was proved by A. MAYER,¹ who also established a relationship between diffusibility and food-value.

Additional sources of nitrogen were found in *amido-acids* and substances of similar composition—*allantoin*, *guanine*, *uric acid*, *acetamide*, and *asparagine*; whilst *kreatine* and *kreatinine* were of less value in this respect. Other nitrogenous substances proved to be unsuitable, such as, *e.g.*, *caffeine* and *hydroxylamine*. *Saccharomycetes* were unable to assimilate *salts of nitric acid*, though a *mucor* was easily able to do so (FITZ²). *Nitrites* are directly injurious (LAURENT³).

A. Mayer has proved that this nitrogen is not only applied to the production of new albumin, but that an actual metabolism of nitrogen occurs, by the fact that the available nitrogen is really consumed, and that yeast-fungi in culture-media which contain less assimilable nitrogen are, *ipso facto*, injured as regards their vital activity and fermentative capacity. The amount of nitrogen in the yeast becomes gradually less, and that not only by its distribution throughout a greater number of cells, but also *absolutely*—*i.e.*, the yeast excretes into the surrounding media a nitrogenous substance which cannot, to its full extent, again be assimilated. The nature of this excretory product has not yet been determined.

In addition to carbon and nitrogen, yeast naturally also requires *nutrient salts*; this postulate, too, was satisfied by Pasteur, who proved that sugar and ammonium salts were unable *by themselves* to supply the wants of yeast, though an addition of *yeast-ash* was quite sufficient for the purpose.

The question of *which* salts are indispensable to the yeast has been very fully discussed by A. MAYER,⁴ but we will only take the facts from his description.

¹ For further particulars on this subject and for bibliography, see A. Mayer, *Gährungschemie*, 124 *et seq.*

² Fitz, *Ber. d. d. chem. Ges.*, viii., 540, 1875.

³ Laurent, *Ann. de la Soc. Belge de Microscopie*, xiv. (Koch's *Jahresb. üb. Gährungsorg.*, 54, 1890). He gives a full description of the nutrition of yeast, to which I can only refer here.

⁴ A. Mayer, *loc cit.*, 137 *et seq.* (also gives the literature).

According to him, the following salts are indispensable:—*Iron*, *potassium* (which cannot be replaced by *sodium*), *magnesium*, *phosphorus*, and very probably *sulphur*, though not in the form of *sulphuric acid*. On the other hand, *calcium* and *sodium* are not essential.

Water is also obviously a necessary food material of yeast. WIESNER¹ found that yeast containing from 40 to 80 per cent. of water was capable of living and producing fermentation, and that it could resist being slowly dried. By rapid variations in the concentration, with the natural result of sudden changes in the osmotic pressure, the yeast-cells may be injured and even killed.

The question of the influence of the concentration of the *solution of sugar* to be fermented is obviously most intimately connected with the question of the amount of water in the yeast. The optimum is 8 per cent., and at about 35 per cent. the fermentative capacity becomes very weak. In like manner, concentrated solutions of glycerin and of salts act prejudicially by withdrawing the water. *Mucor-yeasts* cannot well bear a concentration of more than 7 per cent.²

In these culture-media, then, the yeast-cell exercises its vital functions, it *assimilates*—builds up its cell structure from *carbon*, *nitrogen*, *nutrient salts*, and *water*—and also distributes in *regressive metabolism*, in which process, as we have observed, nitrogenous end-products, of whose constitution nothing more is yet known, are produced, in addition to the carbon compounds, which we cannot, of course, describe with absolute certainty as *metabolic products*. Within its cell it further produces its *specific ferment*, *zymase*, and by means of this fulfils its function, the *decomposition of sugar into alcohol*.

The *reproduction* of the yeast also obviously belongs to its vital functions. So long as the yeast-cell has sufficient nourishment at its disposal its total volume increases, chiefly, under normal conditions, by *budding*. The amount of new yeast formed stands normally in an approximately definite ratio to its fermentative function as measured by the quantity of *alcohol* produced. According to A. MAYER,³ this new growth amounts to 1.38 to 2.03 per cent. of the dry substance of the yeast.

A. BROWN⁴ asserts that the final number of yeast cells depends almost exclusively on the composition of the culture-medium

¹ Wiesner, *Wiener Acad. Sitzb.*, lix., [II.], 495, 1869.

² Quoted by Flügge, *Micro-organismen*, 1896, 229.

³ A. Mayer, *loc. cit.*, 119.

⁴ A. Brown, *Journ. Chem. Soc.*, lxi., 369, 1892.

and but little on the quantity of cells originally introduced. When he placed eight times as many cells in one culture-medium as in another of the same composition, the number of cells in each was almost the same at the end of the fermentation. When a certain quantity was reached there was thus no further increase, but even occasionally a partial decomposition.

We must here again return to the question of such theoretical importance—whether really the vital processes are invariably inseparably connected with the fermentative processes.

If we regard the resulting *by-products* as non-fermentative products of metabolism, we have then, of course, as we have pointed out above, sufficient grounds, in their conditions of development and especially in their quantitative relationship, for concluding that there is merely a *parallelism* between the two processes. But, besides, on a closer study of the *pure alcoholic fermentation* certain reasons in support of our view stand forth prominently.

Thus, MACH and PORTELE¹ found that the *vegetative* and *fermentative* energy did not reach their maximum simultaneously, but that the yeast at first increased more rapidly than it caused fermentation, and that not until a later period was the maximum of fermentative action reached.

Moreover, the fermentative activity does not immediately stop, when the yeast ceases to develop from the want of assimilable material. According to our view there is then still a certain amount of ferment present, which must first be used up.

The contrast is also interesting, that whilst the *assimilability* of proteid substances by the yeast largely depends upon their diffusibility,² it is not possible to establish a relationship between the *velocity of fermentation* of the sugar and the diffusibility.³ Further, when we examine the action of different physical and chemical agents on the yeast, we find that although, in general, there is a very close agreement between the behaviour of the living yeast and of the ferment, there are yet some few observations which indicate that the fermentation is not *completely* dependent on the life of the organisms.

It is true that such an easy means of differentiation as we find in the case of ordinary enzymes is not possible with *yeast zymase*; we can readily demonstrate the existence of its *invertase*, &c., by destroying with certain poisons the vital

¹ Mach and Portele, *Landw. Versuchsstat.*, xli., 261, 1892.

² A. Mayer, *loc. cit.*, 129.

³ Gayon and Dubourg, *Comptes Rendus*, cx., 685, 1890.

energy of the cells; but if we attempted to do the same with the alcohol-producing ferment we should soon be convinced that without exception all the agents which injured the *vital functions* of the yeast also injured its *fermentative energy* to the same extent. As soon as the parent cell is killed or severely injured, the very unstable ferment is also very rapidly destroyed.

The fact that high temperatures and strong acids and bases destroy the cell with the ferment is also in complete analogy with the ordinary enzymes.

As soon, however, as we try the *protoplasm-poisons* to which ordinary enzymes are nearly indifferent, the condition of affairs is completely changed. All those poisons which destroy the living cells, also interfere with the fermentative function of the yeast.

Of the substances which are thus used for the demonstration of the *enzymes of yeast*, we need only mention here *chloroform*, *toluene*, and *mercuric chloride*. On the other hand, many salts—*e.g.*, those of *iron* and *manganese*—are not injurious; whilst others—*e.g.*, *potassium cyanide* and *sodium sulphide*—are markedly so. *Strychnine* and *quinoline* are harmless; *quinine* slightly injurious.¹ *Aluminium salts* have even a stimulating effect, as also *phosphoric acid* and its salts, and *asparagine* (EFFRONT²).

Sodium arsenate has but little influence upon yeast, but considerable action upon other fission-fungi (SCHÆFFER and BÖHM³).

Special attention has been given by SCHULZ⁴ and his pupils to the investigation of the influence of *poisons* upon yeast. They found that small doses, *e.g.*, of *formic acid*, &c., stimulated its action, but that larger quantities were injurious.

As regards the action of metallic salts upon yeast, MANN⁵ concludes that there is a combination between them and the phosphates in the cell.

Carbon dioxide has a restrictive influence (FOTH⁶). *Sulphur dioxide* in strong solutions (200 c.c. of gas to 1 litre of water) kills yeast very rapidly (LIHOSSIER⁷), specially in acid fluids.

Sodium fluoride, which, as is well known, does not fail to destroy putrefactive bacteria, when its solution contains about 1 per cent., has a weaker action upon yeast, and hence its use, as proposed by Effront, has been advocated in breweries to exclude bacteria. In the case of the fluorine compounds we also find one of those instances mentioned above, in which there appears to be a certain difference between the purely vital and

¹ For bibliography and further particulars see A. Mayer, *loc. cit.*, 147.

² Effront, *Bull. Soc. Chim.* [3], ix., 151, 1893.

³ Schæffer and Böhm, *Sitzb. d. Erlanger phys. med. Soc.*, N.S., iii., 238, 1872.

⁴ Schulz, *Virch. Arch.*, cviii., 427, 1887; *Pflüg. Arch.*, xlii., 517, 1888.

⁵ Mann, *Ann. Inst. Pasteur*, viii., 785, 1894.

⁶ Foth, *Z. ges. Brauw.*, 182, 1889.

⁷ Linossier, *Ann. Inst. Past.*, v., 171, 1891.

the fermentative energy. Thus EFFRONT¹ found that *sodium fluoride* and other fluorine compounds when present in slight concentration checked the reproduction of the yeast-cells, but, on the other hand, not only did not have any prejudicial activity on the action of the ferment, but even stimulated it.

According to FOTH,² yeast behaves in a similar manner in an atmosphere of carbon dioxide.

An analogous instance of the fact that it is possible under certain conditions to preserve the fermentative activity whilst destroying the vital activity, is furnished by the experiments of FIECHTER,³ who found that hydrocyanic acid, even in very slight quantities, destroyed the vital activity of the yeast without immediately putting an end to the fermentation.

The question so often raised in discussing the enzymes, and not answered with complete unanimity, as to how far the *products of the decomposition* have an injurious action, receives the simple answer in the case of alcoholic fermentation, that here one of the decomposition-products—viz., *alcohol*—is poisonous to the protoplasm in certain degrees of concentration, and thus injures both the yeast and the fermentation. 12 per cent. of alcohol prevents the growth of *saccharomycetes*, and 14 per cent. destroys all manifestation of energy. *Mucor yeasts* terminate their activity when the alcohol is as little as $3\frac{1}{2}$ to 4 per cent., *M. stolonifer* even with 1.3 per cent.⁴ There are, however, also *saccharomycetes* which are very sensitive towards alcohol, as, for example, a beer-yeast of the Saaz type, examined by PRIOR,⁵ and the alcohol-producing mould-fungi of *koji-yeast* (*vide supra*).

The Significance of Oxygen and Pasteur's Theory of Fermentation.—The study of the fermentative and vital processes of yeast under the influence of the admission of a greater or smaller *amount of oxygen* has furnished further proofs in support of the view that the alcoholic fermentation of sugar is not under all conditions inseparably connected with the vital process. The discussion of this question appears the more important, inasmuch as it was on the ground of this influence of oxygen that the only real *theory* of alcoholic fermentation was based by Pasteur and upheld by the supporters of the vitalistic view. We must, therefore, deal with this point more fully. In the first place we can admit as an uncontested fact that the yeast-fungus requires

¹ Effront, *Bull. Soc. Chim.* [3], v., 148, 476, 731; vi., 786, 1891.

² Foth, *C. f. Bakt.*, i., 502, 1885.

³ Fiechter, *Ueb. d. Wirkg. der Blausäure*, Diss. Basle, 1875.

⁴ Quoted by Flüge, *Micro-organ.*, 229, 1896.

⁵ Prior, *Centralbl. f. Bakteriöl.* [II.], i., 432, 1895.

for its *fructification* an *unlimited supply of free oxygen*, as was proved beyond doubt by REESS.¹

But there is no such general agreement in the answer to the question of how far the purely *vegetative* energy of the yeast-cell, including its reproduction by *budding*, and its *fermentative action* is affected by differences in the amount of oxygen admitted.

Pasteur made the discovery, so far-reaching in its influence upon his theory, that the yeast-fungus showed very *energetic reproduction* when *abundance of oxygen* was admitted, but that, on the other hand, its *fermentative capacity* was relatively less; and *vice-versâ* when the supply of oxygen was *excluded* altogether, or nearly so, there was less reproduction, but much *more active fermentative capacity*. Pasteur made use of this behaviour of the yeast in the inductive construction of his theory of alcoholic fermentation. He asserted that alcoholic fermentation was "*life without air*" (*vie sans air*), i.e., that the yeast being to a certain extent in a confined condition derived the oxygen required for the development of its vital energy from the sugar, and from it produced carbon dioxide in its organism, whilst it excreted the non-utilised residue as alcohol. This is Pasteur's theory, which we will criticise presently; but first of all we must deal with the actual facts.

Pasteur had proved that an excess of *aëration* promoted the reproduction of the yeast; he concluded, without further proof, that the oxygen was the natural agent in this phenomenon. Experiments made by NEUBAUER² and by HANSEN,³ however, showed that "*aëration*" with other gases, and even shaking, without the introduction of oxygen, produced similar results. It thus appeared that the *oxygen*, as such, was not the direct cause of the reproduction.

On the other hand, Pasteur's experiments received support from BREFELD,⁴ who carried to an extreme conclusion Pasteur's view that fermentation proceeded best in the absence of oxygen, and the life and development of the yeast when it was present; but from this material he sought to forge a direct weapon against Pasteur's vitalistic *theory*. Brefeld first endeavoured to prove that free oxygen was indispensable to the *vital process* of the yeast; he attempted to demonstrate that *phenomena of death*

¹ Reess, *Botan. Unters. üb Alcoholgährungspilze*, 15.

² Neubauer, *Ann. d. Oenolog.*, iv., 68.

³ Hansen, *Meddelelser fra Carlsberg Labor.* Quoted by Mayer, *loc. cit.*, 153.

⁴ Brefeld, *Verh. phys. med. Soc. Würzburg*, 163, 1873. *Ber. d. d. chem. Ges.*, vii., 281, 1066; viii., 421, 1875. *Thiel's Landw. Jahrb.*, iii., 1876.

could be directly recognised in the yeast under the microscope; he further tried to prove that the yeast-cells possessed a great affinity for oxygen, and that they could consume to the last trace the oxygen in culture-media containing but little of that element. So long, then, as oxygen was present, the yeast-cell lived and budded in its normal vital process; but as soon as the oxygen *disappeared*, the growth *completely ceased*, and then the *fermentation* began. According to Brefeld, *fermentation* and *growth* never occurred simultaneously; the one excluded the other. Fermentation was a *morbid* process ending with the death of the yeast. To this extent he opposed Pasteur's view that, although alcoholic fermentation was an adaptation to altered conditions, yet within those limitations it was a normal manifestation of life. But Brefeld lacked proof that the yeast actually did not grow during the fermentation, and that the fermentation only began after the complete disappearance of the oxygen. Brefeld's assertions were very soon after energetically opposed by Pasteur himself, and in particular by MORITZ¹ and TRAUBE.²

Thus Traube proved that yeast did not *develop* in the *absence of oxygen*, even on the best culture-media, but that, on the other hand, *developed yeast* could do without free oxygen.

It was then soon also irrefutably proved that, on the one hand, in the *absence of oxygen* growth occurred in addition to fermentation, and *vice versa*, in the *presence of oxygen* there was fermentation in addition to growth. MAYER³ very lucidly remarks that Brefeld's views are justified, if we conclude that there are two final stages of the process: on the one hand, the very *young* yeast, which, when there is an abundance of oxygen, grows luxuriously *without producing fermentation*; and, on the other hand, *old weak* yeast, which still causes fermentation, when oxygen is excluded, *without undergoing further development*; and that, again, under average conditions both processes occur simultaneously. We are thus justified in forming the conception that, although the introduction of oxygen weakens the fermentative capacity of certain yeast-cells, yet it does not destroy it. In practice, it is found that "aërated" yeast produces *more alcohol in toto* notwithstanding the smaller energy of these *individual cells*, since this deficiency is more than compensated by an increased *production of new cells* (PEDERSEN⁴).

¹ Moritz, *Ber. d. d. chem. Ges.*, vii., 156, 436, 1874.

² Traube, in various articles, *Ber. d. d. chem. Ges.*, vii.-xv.; see also his separate treatise, Berlin, 1899.

³ A. Mayer, *loc. cit.*, 155.

⁴ Pedersen, *Meddelelser fra Carlsberg Labor.*, iv., 1878. Quoted by Mayer, *loc. cit.*, 156.

However, not even the assertion that oxygen weakens the fermentative capacity has remained unchallenged. NÄGELI¹ even claimed to have proved that a direct acceleration was caused by oxygen. But this view of Nägeli has been refuted on further investigation of the question, notably by A. MAYER² and GILTAY and ABERSON.³ The last-named authorities showed by careful experiments that, when there was a vigorous growth accompanying abundant aëration, only 75 per cent. of the sugar was fermented under the conditions specified, whilst, on excluding oxygen, as much as 90 per cent. underwent fermentation. Others again (e.g., A. BROWN⁴) have come to the conclusion that, when *the number of yeast-cells remains constant*, the introduction of oxygen causes a very slight *acceleration* in the production of alcohol. According to BUCHNER and RAPP,⁵ it is almost indifferent under normal conditions, but *injurious* rather than *beneficial*. We must thus, taking everything into account, consider it proved that *free oxygen accelerates the growth of yeast, but only influences the fermentation to a very slight extent*. Particularly pregnant are the experiments of BUCHNER and RAPP,⁶ who were able to detect vigorous fermentation even on cultivating the yeast on plates of gelatine and sugar exposed to the air, so that about $\frac{6}{7}$ of the sugar was still actually *fermented*, and only about $\frac{1}{7}$ *consumed* in the metabolism of the cells. We must now consider what theoretical bearing these facts have upon our views on the nature of fermentative processes in general, and of alcoholic fermentation in particular.

Pasteur's radical conception, and his theory of fermentation deduced from it, is no longer tenable in the light of the results mentioned above. If yeast also *causes fermentation in the presence of oxygen*, it follows that we cannot correctly say that it is the *absence of oxygen* which forces it into an intense transformation of its metabolism in the direction of an alcoholic fermentation of the sugar. All attempts to uphold Pasteur's conception and theory, conditionally and in a limited sense, must be vain. He himself has laid stress, with emphasis, on *one* point. Either alcoholic fermentation is *une vie sans air* or it is not. Compromises are out of the question. Now, the experimental facts are *against*

¹ Nägeli, *Theorie der Gährung*, Munich, 1879.

² A. Mayer, *Landwirthsch. Versuchsstat.*, xxv., 301.

³ Giltay and Aberson, *Pringsheim's Jahrb. f. wissensch. Botanik.*, xxvi., 543, 1894.

⁴ A. Brown, *Journ. Chem. Soc.*, lxi., 369, 1892.

⁵ Buchner and Rapp, *Z. f. Biol.*, xxxvii., 82, 1899 (full bibliography).

⁶ Buchner and Rapp, *Z. f. Biol.*, xxxvii., 82, 1899.

Pasteur; and with this his theory, the only *theory* of the *vitalistic conception*, falls to the ground, and all that remains is the classification of organised ferments under the vital process, which is equivalent to the renunciation of every dynamic explanation.

The overthrow of Pasteur's theory is the first and most important result of our clearer view of the significance of oxygen for the yeast-fungus.

But what do these facts teach us with reference to our dynamic conception of fermentative processes?

They show us with great clearness that there are exceedingly important factors which influence the *vital process* of the yeast in a *different manner* to the *fermentative action*. The free oxygen *increases* the vital processes of the fungi, but *reduces* the production of their ferment.

We conclude that in this process the fermentative capacity is *actually per se* reduced, and that it is not merely a relative diminution in the production of alcohol due to the vigorously-breathing yeast directly utilising and consuming by combustion a relatively greater quantity of the sugar. A direct proof of this important conclusion, which must be based on a parallel determination not only of the alcohol, but also of the carbon dioxide, which, apart from the typical fermentative process, is also possibly produced by respiration, has been sought in vain. A. MAYER¹ only asserts very briefly that in the case of vigorously-growing yeast part of the increased consumption of the sugar is also "the result of a direct oxidation of sugar in the sense of ordinary respiration," without entering into the fundamental question of the ratio of alcohol to carbon dioxide under these different conditions. Since, however, a decision is not possible without experimental investigation, we prefer to assume that the fermentative capacity is actually reduced *per se*, as indeed is *a priori* to be concluded, on account of the great differences. When the supply of oxygen is very abundant there occurs beyond doubt, according to BUCHNER and RAPP,² a perceptible direct oxidation of the sugar.

If we may no longer conclude with Pasteur that the entire vital process of the fungi is so radically changed by the total absence of free oxygen that now in their completely altered metabolism they derive the oxygen essential to their life from the sugar, then the conception—that whilst the rest of the metabolism is *intensified* by variations in the amount of oxygen, the *alcohol-metabolism*, if we may make use of this concise expression, is *reduced* in extent—becomes an assumption which theoretically leads us no further, and as to which serious doubts must be expressed. We have learnt, however, from E. Buchner that the yeast-cell produces an *enzyme*. How much more simply and with greater theoretical significance can we account for the

¹ A. Mayer, *loc. cit.*, 159.

² Buchner and Rapp, *loc. cit.*

influence of the oxygen by the assumption that free oxygen, although, as in the case of all organisms, rendering the entire *vital process*, as such, more active, yet checks the *production of the ferment*.

With this thought we come once more to the conclusion so often arrived at before, that although vital processes and fermentative processes are connected in the sense that actions of ferments are a very important *instrument* for the manifestation of vital processes, yet we are not justified in identifying the two without further proof. Theoretically, they are processes which run parallel and independently of one another.

If we would hold this view we must also deal more closely with the question of what general *physiological significance* this alcohol-producing ferment has for the yeast-cell.

The *ordinary function* of unorganised ferments of forming assimilable soluble products by decomposition from non-utilisable food-substances, is *not* that of the alcohol-producing ferment of yeast. To this extent NÄGELI¹ is completely justified in laying stress upon the difference between alcoholic fermentation and the action of ferments in the narrower sense of the word, although I must dissent from the far-reaching theoretical significance for the nature of fermentative processes in general which he assigns to this difference. We have here a teleological and biological difference, but one which does not come within the scope of a purely theoretical consideration of fermentative processes as a dynamic unit. This, however, does not lessen its importance to a conclusion as to the *physiological function* of ferments. The alcohol ferment of yeast is not a *nutritive enzyme* in the sense given above; for neither the *alcohol* nor the *carbon dioxide* serve as *food-material* for the yeast. The former can lay no claim to the title since the sugar presents a much better form of nourishment; the yeast-cell cannot assimilate the carbon dioxide because it possesses no *chlorophyll*. What then is the physiological function of the ferment of yeast? We can hardly come to any other conclusion than that the ferment has the function of *conveying energy* to the yeast-cells by means of the exothermic reaction which it brings about.

We know from numerous instances that all ferments are, as a rule, only produced when the organism requires them.

So long as, for example, mould-fungi meet with sufficient grape-sugar in their culture-medium they produce no enzymes; and so long as the quiescent embryo of the seed of the plant has

¹ Nägeli, *Theorie der Gährung*, Munich, 1879 (see p. 8)

no need to draw upon the reserve material at its disposal, no production of enzyme occurs.

In the light of these facts we must attempt to also explain the influence of oxygen on the production of the ferment. There are *Saccharomycetes* closely resembling yeast—e.g., *S. mycoderma*, which can only live in the air, *never* produces ferments, and must perish when the air is excluded. It has no means of maintaining its vital energy, when there is a deficiency of oxygen.

This means is at the disposal of other micro-organisms.¹ The *mucor-species* also, as a rule, live aërobically without producing an alcohol ferment. But when they are cultivated *in the absence of air* they acquire the capacity of forming a ferment, which by means of its *exothermic processes* imparts to them the *energy* which they cannot, like the chlorophyll plants, derive from the solar energy conveyed to them, nor, as under normal conditions, produce for themselves in an exothermic process of *respiration*, so long as they lack the necessary oxygen for the process. But the production of the ferment remains to them a *makeshift*, which they immediately dispense with when they regain their normal vital conditions (*admission of oxygen*).

Now the true yeast-fungi are so eminently adapted to this capacity of assisting the purely vital oxidation process by the production of a ferment that they make use of it *even under conditions* in which *free oxygen*, being present, it would be possible for them to do without this aid; with the result that it is not possible to bring them back to an aërobic condition of life devoid of ferments. On the other hand, they are capable, through its free application, of maintaining by its aid their total vital energy in its full extent for a long time, even when every other source of energy is cut off, by the complete *exclusion of oxygen*. As regards the degree in which they make use of this assistance they differ from the members of the *mucor family* which, because they normally live aërobically and without the ferment, are less abundantly endowed with it, so that when they are placed in an *anaërobic condition of life* their vegetative capacities *disappear* with the production of the ferment *more rapidly* than in the case of the *yeasts*; indeed, even with the latter the capacity for anaërobic life, which is only rendered possible through the production of the ferment, is not unlimited. Eventually, when oxygen is excluded, the yeast cell also dies after the ferment has become exhausted.

¹ As regards the question of anaërobiosis, and the action of ferments in general, see Liborius, *Z. f. Hyg.*, i., 115, 1886-7.

Again, when there is a deficiency of substratum for the fermentation, the yeast must ferment part of its own substance to furnish vital energy for the remainder, as is the case in the *auto-fermentation* of yeast mentioned above.

In this way, starting from totally different theoretical principles, we arrive at *physiological* conclusions similar to those formed by Pasteur. The production of the ferment is the *physiological substitute for free respiration* in the production of the necessary vital energy, but the metabolism, in the absence of oxygen, is not the *fermentation itself*. Apart from our general conception of fermentative processes this latter view has been rendered untenable by the discoveries of E. Buchner.

As in the case of the yeast cell we can, in like manner, also look for a dynamic explanation of the production of alcohol, which occurs in the organs of *higher plants*, and presumably also of *animals* (*vide supra*), when oxygen is excluded. The production of ferments which induce exothermic processes appears to be a medium of universal occurrence, by the aid of which the necessary vital energy is maintained, if only for a time, even in the absence of oxygen. Possibly the *production of lactic acid* in animal organs, which occurs when there is a large *consumption of oxygen*—*e.g.*, in *working muscle* and in *phosphorus poisoning*—is to be accounted for in a similar manner.

CHAPTER XXIII.

THE OXYDASES.

FROM the results of investigations which, for the most part, have been made in recent years, oxidising ferments, *oxydases*, which act as carriers of oxygen, have a great rôle assigned to them in nature. Like the *hydrolytic* ferments, they appear to fulfil an important function in the metabolism of living organisms.

The Oxydases.—We will first discuss the oxidising ferments of animal tissues. The problem of the oxidation of combustible substances in the animal organism, connected as it is in the most intimate fashion with the nourishment, naturally occupied the attention of physiologists, even at an early period. And soon the remarkable phenomenon was observed—that the animal organism, which was able to decompose, without high temperatures and energetic chemical agents, substances so extremely difficult to oxidise as the *proteids*, could also transmit unchanged through its tissues easily oxidisable substance, as, *e.g.*, *oxalic acid*.¹ And when, in particular, the decomposition of food-materials had come to be attributed less to a vital decomposition process than to the action of the unorganised ferments of the intestinal tract, there were still left examples enough to show that the living organism possessed the property of oxidising not only readily combustible *autoxidisable* substances, but also such as, outside the organism, offered great resistance to the action of oxidising agents—*i.e.*, *disoxidisable* substances (TRAUBE). *Benzene* leaves the body as *phenol*, *toluene* as *benzoic acid* or *hippuric acid*,² &c.

Explanations of these facts were naturally sought. HOPPE-SEYLER³ based his view upon the fact that strong *reduction*—

¹ Pohl, *A. f. exp. Path.*, xxxvii., 413. See also Hahn, *Berlin. klin. W.*, 499, 1897.

² Naunyn and Schultzen, *Dubois A.*, 349, 1867. Baumann and Herter, *Z. physiol. Ch.*, i., 265, 1897.

³ Hoppe-Seyler, *Ber. d. d. chem. Ges.*, xvi., 117, 1917, 1883.

processes occurred in the tissues, and could be detected¹ by the use of alizarine blue and other colouring matters,² as proposed by EHRLICH.³ From this, Hoppe-Seyler formed the conception that in such processes of reduction *molecular oxygen*, O_2 , was rendered active by conversion into O , just as, e.g., palladium foil, which is saturated with hydrogen, decomposes molecular oxygen, so that palladium can oxidise, e.g., indigo. In his opinion, similar stimulating processes occurred in the organism itself under the influence of reduction. He regarded readily-oxidisable substances as *conveyors of oxygen*.

This view of Hoppe-Seyler was discussed, notably by TRAUBE,⁴ in several publications. He replaced it by the notion of a *catalytic oxidation*, a *stimulation* of the oxygen, as is assumed to be the case in the ready liberation of oxygen from hydrogen peroxide on contact with many chemical substances. He showed that in the slow oxidation of *autoxidisable*—i.e., easily oxidised—substances, a *rendering active* of molecular oxygen did not occur, and that, lastly, free oxygen atoms did not possess the power of oxidising *disoxidisable* substances to the extent ascribed to it by Hoppe-Seyler. As a matter of fact, it was shown by SCHÖNBEIN⁵ and A. SCHMIDT⁶ that animal and vegetable tissues contained such catalytically-active substances, capable of liberating from hydrogen peroxide free oxygen, which, e.g., turned *guaiacum tincture* blue—which molecular oxygen did not do. This catalytic action, however, we now also assume to be due to enzymes, and, in fact, Traube has already formed the conception of an *oxidation ferment* for this phenomenon.

As a matter of fact, enzymic substances appear to be the active agents here. These oxidation processes have not the close connection with reduction which Hoppe-Seyler ascribed to

¹ These processes, of course, have now begun to be attributed to independent *enzymes*, so that we shall soon be presented with *reducases* in addition to *oxydases* (cf. ABELOUS and GÉRARD, *Comptes Rendus*, cxxix., 164, 1899). That such a *reducing ferment* must induce an *endothermic* process, accompanied by an absorption of energy, and thus directly contradict the definition of a ferment, does not concern M. Abelous. To this category also belongs the *philothion*, the "reducing" *hydrogenising* ferment of REY-PAILHADE (*C. R. Soc. Biol.*, 1894, 1895, 1897).

² See also Harris, *Journ. of Anat. and Physiol.*, xxxi., 381.

³ Ehrlich, *Das Sauerstoffbedürfniss des Organismus*, Berlin, 1885.

⁴ Traube, *Ber. d. d. chem. Ges.*, xv., 659, 2421, 2434; xvii., 123, 1201. See also the separate publication, Berlin, 1899.

⁵ Schönbein, *Z. f. Biol.*, i.-iv. Cf. Schaar's survey of Schönbein's researches, *Z. f. Biol.*, xxxvii., 1899.

⁶ A. Schmidt and others, *Pflüg. A.*, vi., 508. See also on this point Pflüger in his *Arch.*, x., 252 et seq.

them. For, according to the results of SPITZER,¹ the glycolytic extracts do not have a reducing action; then the reducing capacity of the tissues increases after death, whilst the *oxidising* capacity soon disappears; but, besides this, the reducing capacity is retained even after boiling, whereas the oxidising ferment is immediately destroyed by it.

The methods by means of which we can make a qualitative and quantitative investigation of these ferments are as follows:—

The oxidising action can be recognised, in particular, by means of the formation of *indophenol*. When the *bruised tissue*² or *extracts of organs*³ are mixed with an alkaline solution of a mixture of *α-naphthol* and *p-phenyldiamine* (or di- or tetramethyl-para-phenylenediamine⁴), a blue coloration results on the absorption of oxygen, due to the formation of indophenol, as was first observed by EHRLICH.⁵ RÖHMANN and SPITZER then found that the bruised tissue of organs was also able to bring about similar synthetic oxidations, for which two atoms of oxygen were required—*e.g.*, those of *indamines* and *eurhodines*.

Other colour reactions are the formation of a blue colour in *guaiacum tincture*, the dark-brown coloration of *p-phenylenediamine*, and the garnet-red coloration of *guaiacol* (BOURQUELOT⁶).

SCHAAR⁷ considers that the blue coloration of guaiacum is due to *guaiaconic acid*, which forms a blue compound with *ozone*.

Another method of quantitatively determining the activity of the enzymes is due to SCHMIEDEBERG⁸ and his pupils. They allow the substances under examination to act upon *salicylic aldehyde* or *benzyl alcohol*, and determine the amounts of *salicylic acid* (colorimetrically with ferric chloride) or *benzoic acid* produced. POHL⁹ also uses *formaldehyde*, which is oxidised to formic acid; SPITZER¹⁰ *arsenious acid*, which produces *arsenic acid*.

The most suitable appears to be *salicylic aldehyde*.

The enzymes in question appear to vary in their nature.

¹ Spitzer, *Berl. klin. Woch.*, 949, 1894.

² Röhmann and Spitzer, *Ber. d. d. chem. Ges.*, xxviii., 567, 1895. Spitzer, *Pflüg. A.*, lx., 303.

³ Pohl, *A. f. exp. Path.*, xxxviii., 65.

⁴ Wurster, *Ber. d. d. chem. Ges.*, xix., 3195, 1886.

⁵ Ehrlich, *loc. cit.*

⁶ Bourquelot, *C. R. Soc. Biol.*, xli., 896, 1896.

⁷ Schaar, *Apotheker-Zeitg.*, 749, 1894.

⁸ Schmiedeberg, *A. f. exp. Pathol.*, xiv., 288, 379. Jacquet, *ibid.*, ix., 386.

⁹ Pohl, *ibid.*, xxxviii., 65.

¹⁰ Spitzer, *Pflüg. Arch.*, lxxi., 596.

In the first place we must clearly ascertain which of all these reactions can really be attributed to enzymic action.

BOURQUELOT,¹ to whom we are also indebted for much experimental material on this question, concludes that in all these catalytic oxidations there is a transference of oxygen. All these processes, therefore, can *only* occur in the presence of free oxygen.

He classifies these oxidising substances in four groups.

The *first* is occupied by *ozone* alone, which has an inherent power of conveying oxygen, and of *turning guaiacum tincture blue*.

The *second* group comprises the *ozonides* or ozone-carriers of Schönbein as, *e.g.*, *quinone*, which, owing to the active ozone it contains, gives for a time in an aqueous solution all these colour reactions, but immediately loses this oxidising power on contact with many organic substances—*e.g.*, *milk, blood, &c.*—as also on *heating* and standing.

Bourquelot's *third* group comprises the true *oxydases*, which can be distinguished from the second group by the fact that their activity is not dependent on a definite quantity of an oxidising agent like ozone, since, being *true ferments*, they continue to convey oxygen until their activity is put an end to by the agents which destroy ferments, notably by *boiling*.

Lastly, he differentiates as the *fourth* group such substances as produce oxidising reactions in the presence of *hydrogen peroxide*, and only then, since they use the hydrogen peroxide as the source of the oxygen to be conveyed. Such substances are also of frequent occurrence in vegetable and animal secretions. ABELOUS and BIARNÈS² term them *indirect oxydases*. Their activity also is destroyed by boiling. Hydrogen peroxide is also *decomposed* by the *true oxydases* as by all ferments, but their oxidising action is not inseparably connected with it. Moreover, the genuine *ferment* here is also probably distinct from the principle which *decomposes hydrogen peroxide* (BOURQUELOT³).

Hence, before an oxidation of this kind can be attributed to a true "oxydase" the possibility of its being an *ozone reaction* must first be eliminated; and secondly, the substratum must be free from *hydrogen peroxide*, with the aid of which *indirect oxydases* might also cause oxidation.

It is not yet possible, however, following this rule, to attribute

¹ Bourquelot, *C. R. Soc. Biol.*, 402, 1897.

² Abelous and Biarnès, *C. R. Soc. Biol.*, 495, 1898.

³ Bourquelot, *C. R. Soc. Biol.*, 381, 1898. Cf. Jacobson, *Z. physiol. Chem.*, xvi., 340.

with certainty all the recorded oxidation processes in animal and vegetable tissues to the action of true oxydases ; indeed, the whole question is theoretically still so little ripe for discussion that we will content ourselves with giving a summary of what has been discovered experimentally. Of the animal oxydases *salicylase* (ABELOUS), which oxidises salicylic aldehyde, has been the most thoroughly investigated.

In the living organ the enzyme is attached to the cells ; it can, therefore, be detected in the organ immediately after death, and in the freshly-bruised tissue of the organ (JACQUET¹). On the other hand, glycerin or chloroform and water extracts of the *living* organs are inactive, though active extracts are readily obtained from the *dead* organs (PÖHL²).

It does not give the *indo-phenol* reaction.

The oxidising capacity of surviving organs is not interfered with by either *poisons* or *freezing* (JACQUET). Only *hydrocyanic acid* and *hydroxylamine* have a restrictive influence (RÖHMANN and SPITZER³). *Alcohol* in the concentration of 80 per cent. does not injure the ferment, and hence the organ can be treated with such alcohol, and the ferment precipitated with it and kept in a dry condition ; it remains active. Alcohol of 96 per cent. strength, however, appears to destroy it slowly (SCHWIENING⁴), as do also *alkalies* and *acids* (SPITZER). It is readily soluble in water. It acts best at 60° C. ; at 100° C. it is very rapidly destroyed (ABELOUS and BIARNÈS⁵). Abelous and Biarnès have also proved experimentally that during the action of the ferment there is an absorption of oxygen and a liberation of carbon dioxide.

As regards the activity of different organs the following statements have been made:—The blood, according to JACQUET,⁶ contains no oxydase, but this is disputed by SALKOWSKI⁷ and by ABELOUS and BIARNÈS.⁵ According to the last-named authorities, the *muscle*, *nerves*, and *pancreas* have practically no action, but the *liver*, *lungs*, and *spleen* a very energetic one. Abelous states that the organs of *young animals* contain more

¹ Jacquet, *A. f. exp. Pathol.*, xxix, 386.

² Pohl, *A. f. exp. Pathol.*, xxxviii., 65.

³ Röhmman and Spitzer, *Ber. d. d. chem. Ges.*, xxviii., 567, 1895.

⁴ Schwiening, *Virch. A.*, cxxxvi., 478.

⁵ Abelous and Biarnès, *Arch. d. Physiol.*, 195, 239, 1895 ; *C. R. Soc. Biol.*, xlviii., 97, 262.

⁶ Jacquet, *A. exp. Path.*, xxix., 386.

⁷ Salkowski, *Z. physiol. Ch.*, vii. *Centralbl. med. Wiss.*, 913, 1894. *Virch. Arch.*, cxlvii., 1.

ferment. JACOBY¹ has investigated *salicylase* in various ways. He found that it was without action upon sodium thiosulphate, acetic acid, and stearic acid. The last experiment was made with reference to the possible formation of sugar from fat by oxidation, which is said to occur in the body.

Chloroform in small quantities was found to have a stimulating effect, but in greater concentration was injurious. On the other hand *sodium carbonate* proved to be extremely injurious, a 1 per cent. solution of it preventing the action of the ferment, and *sodium hydroxide solution* was still more prejudicial.

He has recently attempted to isolate the *salicylase* of the liver in the following manner:—The livers of oxen were triturated with quartz sand, and the extracts fractionally precipitated with ammonium sulphate. The precipitate was extracted with water, precipitated with alcohol, again extracted, and precipitated with uranium acetate. The preparation gave neither the biuret nor Millon's reaction. The ferment was destroyed by boiling and by free acids and alkalies.

MEDWEDEW² considers that he is justified, even at this stage, in bringing the action of the ferment within the bounds of mathematical formulæ. Thus, he asserts that "the quantity of salicylic acid formed in 1 volume-unit is proportional to the square of the concentration of the oxidation-ferment, and inversely proportional to the square-root of the concentration of the salicylic aldehyde." In my opinion it is not difficult to form a correct estimate of such speculations. They are based on such uncertain premises, and the action of the ferment is far too subject to very uncontrollable influences, for a strictly arithmetical conception of these factors to be formed as yet. Above all, the idea conveyed by *concentration* of the ferment is exceedingly vague. Naturally it is much more likely that the concentration of the medium has an influence.

Besides this salicylic aldehyde-oxidising ferment, there are other ferments in the bodies of animals.

According to the results which have been published up to the present, we can practically differentiate the following enzymes:—

I. The *salicylase* of which we have been speaking, which oxidises salicylic aldehyde to salicylic acid. Doubtless it is identical with the agent that oxidises *benzyl alcohol*, *formaldehyde*, and *arsenious acid*.

To the same group probably belong the oxidising principles which SPITZER³ attributes to the *nucleo-proteids*.

¹ Jacoby, *Virch. A.*, clvii., 235, 1899. *Z. physiol. Ch.*, xxx., 135.

² Medwedew, *Pflüg. Arch.*, lxx., 249, 1897.

³ Spitzer, *Pflüg. A.*, lxxvii., 615.

II. The agent, only found in the liver and spleen or their extracts, which acts specifically on *purine* derivatives. After twenty-four hours' digestion with these extracts, with precautions to exclude putrefaction, SPITZER¹ succeeded in converting *xanthine* (oxypurine) and *hypoxanthine* (dioxypurine) almost quantitatively into *uric acid*, which is a *trioxypurine*. *Adenine* and *guanine* were only partially oxidised.

III. The third main group comprises the oxydases which turn *guaiacum tincture blue*, but have no action upon *salicylic aldehyde* (ABELOUS and BIARNÈS²).

They are akin to the *vegetable oxydases* to be described presently.

Their type is the so-called *globulin-oxydase* of ABELOUS and BIARNÈS,² which is insoluble in water. They found it in *blood*, in fresh saline solutions of fibrin, in the residue from artificial trypsin and papain digestions, the filtrate from which proved inactive, and in various *organs*. They regard it as an enzyme in close combination with globulin, like TRAUBE'S³ oxidation-ferment with *myosin*. According to PORTIER,⁴ however, it is not connected with fibrin, but originates with the *leucocytes*.

Similar oxydases turning *guaiacum tincture blue* were discovered by GIARD⁵ in two *ascidiæ*; by PIÉRI and PORTIER⁶ in the blood, antennæ, and gills of molluscs (*Artemis exoleta* and *Ostrea edulis* [*Oyster*]); by ABELOUS and BIARNÈS⁷ and HUGOUNENCQ and PAVIOT⁸ in crabs; and by BIEDERMANN⁹ in the intestinal secretions of the meal-worm (*Tenebrio molitor*).

CARNOT¹⁰ found a similar enzyme in the saliva of *all human beings* and of some animals, *e.g.*, in that of the *dog*, as well as in the secretion of the nose, in pus, and in tears. He did not find it in the urine, bile, or intestinal secretions, but detected traces of it in milk.

There is an uncertainty as to the position of many oxidising agents which act either on artificially-added colouring-matters (*p*-phenylene-

¹ Spitzer, *Pflüg. A.*, lxxvi., 192, 1899.

² Abelous and Biarnès, *C. R. Soc. Biol.*, 285, 493, 559, 576, 1897; 495, 1898. *Arch. d. Physiol.*, 664, 1898.

³ Traube, *Ber. d. d. chem. Ges.*, xv., 659, 1882.

⁴ Portier, *C. R. Soc. Biol.*, l., 452, 1898.

⁵ Giard, *C. R. Soc. Biol.*, xlviii., 483, 1896.

⁶ Piéri and Portier, *Comptes Rendus*, cxxiii., 1314. *Arch. d. Phys.*, 61, 1897.

⁷ Abelous and Biarnès, *C. R. Soc. Biol.*, 175, 249, 1897.

⁸ Hugounencq and Paviot, *C. R. Soc. Biol.*, xlviii., 1896.

⁹ Biedermann, *Pflüg. Arch.*, lxxii., 156, 1898.

¹⁰ Carnot, *C. R. Soc. Biol.*, xlviii., 552, 1896. See also Dupouy, *Journ. Pharm. Chim.* [6], viii., 551; *Maly's Jb.*, 729, 1899.

diamine alone or with α -naphthol, &c.) or upon natural chromogenic substances, and which also have an action upon *phenols* and *tyrosine*. *Tyrosinase*, with which we shall deal more fully when describing the vegetable oxydases or a similar enzyme, has only been found in the animal kingdom by BIEDERMANN,¹ in the intestinal secretion of *Tenebrio molitor*. A ferment which colours a naturally-occurring chromogen was found by PHISALIX² in the skin of the frog.

They appear, however, to belong to the oxydases which turn guaiacum blue; and, on the other hand, Carnot (*loc. cit.*) was also able to observe a violet coloration of *p*-phenylenediamine in the case of the enzymes discovered by him. He also, however, observed the oxidation of *hydroquinone*, which is regarded as a function of Bertrand's *laccase* (*vide infra*).

The decomposition of *fats*, and also of *free palmitic acid*, by a mixture of blood and extract of liver has been affirmed by WEISS,³ but denied by BLUMENTHAL.⁴ In this process *sugar* is said to be produced—a fact which would be of great importance to the supporters of the view that part of the sugar in diabetes is derived from *fats*. The disappearance of fats in the circulation of the blood has been closely studied by COHNSTEIN and MICHAELIS.⁵

Indirect Oxydases—*i.e.*, those which only cause oxidation in the presence of *hydrogen peroxide*—are equally widely distributed, *e.g.*, in serum, which contains no true oxydase; in milk, &c. (see BOURQUELOT⁶). It must not be overlooked that *old guaiacum tincture* frequently contains *hydrogen peroxide*, since, otherwise, true oxydases may be mistaken. It is, therefore, essential to always employ fresh solutions. Indirect oxydases were found by ABELOUS⁷ in various tissues, and by LÉPINOIS⁸ and others in *extracts of liver*.

LINOSSIER⁹ found an oxydase of this kind which turned guaiacum tincture blue, in pus. KLEBS¹⁰ was the first to call attention to the fact that guaiacum tincture was turned blue by pus. Chloroform, hydrocyanic acid, &c., do not destroy the activity of the ferment. Similarly, the chloroform and water extract of the precipitate obtained with alcohol was found to be active.

We must still spare a few words for SPITZER's nucleo-proteids (*vide supra*). Spitzer ascribes to the nucleo-proteids—*i.e.*, the specific substances of the cell-protoplasm—an importance as oxy-

¹ Biedermann, *Pflüg. A.*, lxxii., 152, 1898.

² Phisalix, *C. R. Soc. Biol.*, l., 793, 1898.

³ Weiss, *Z. phys. Ch.*, xxiv., 542.

⁴ Blumenthal, *Z. f. physik. u. diät. Therapie*, 250, 1898.

⁵ Cohnstein and Michaelis, *Pflüg. A.*, lxx., 473; lxx., 76; Summary in *Medic. Woche*, No. 15, 1900.

⁶ Bourquelot, *C. R. Soc. Biol.*, 402, 1898.

⁷ Abelous, *C. R. Soc. Biol.*, 328, 1899.

⁸ Lépinos, *C. R. Soc. Biol.*, 428, 1899.

⁹ Linossier, *C. R. Soc. Biol.*, 373, 1898.

¹⁰ Klebs, quoted by Linossier.

gen conveyors extending beyond life, and believes that this is to be attributed to the peculiar state of combination of the *iron* in these substances. It is not clear whether he assumes that there is an actual combination of the oxygen and a subsequent liberation—*i.e.*, a metabolic process of oxidation and reduction, as in the case of the colouring matter of the blood—or prefers to attribute it to the action of a ferment. In either case his results, taken in connection with the impossibility of obtaining other ferments in a pure condition, are very noteworthy and of the greatest theoretical importance for the further consideration of ferments and processes allied to them, the more so since other ferments (*pepsin*, *diastase*) have been claimed to be nucleo-proteids. What really is the case here, and whether there is possibly a sort of intermediate link between the true fermentative and other allied processes to which the phenomenon is due, cannot, for the present at least, be decided. This result is also worthy of great attention from a biological point of view. It enables conclusions to be drawn as to the function of the nucleo-proteids in the living cell or in its nucleus. It is also possible, on these grounds, to claim an important part in the oxidation processes of the cell for the living nucleus.

Yet it is surely going too far to remove the nucleus from the lofty pedestal on which, by general consent, it is enthroned, and to degrade it to a simple instrument of oxidation, or, as it were, a *force-machine* of the cell, as JACQUES LOEB¹ wishes to do.

Whilst, hitherto, the fact that protoplasmic fragments devoid of nucleus are incapable of development has led to the conclusion that the nucleus is essential for the reproduction of life, LOEB believes that he is justified in ascribing this deficiency solely to an *insufficient exchange of oxygen* due to the absence of the nucleus. In support of this view he has pointed out that he was able to keep alive for a long time (5 to 6 weeks) fragments of algae containing no nucleus, but capable of *assimilating*, by reason of their chlorophyll, and producing oxygen, whereas *infusoria* which contained no nuclei speedily perished. He has, further, come to the conclusion that the distance between two nuclei cannot exceed a certain maximum without the intervening protoplasm being “suffocated.” This is not the place to further elaborate this question, as all I wish to do is to place the significance of Spitzer’s results in the proper light.

The “Urea-producing Ferment.”—A study of the question of the “urea-producing ferment” furnishes an unmistakable proof

¹ J. Loeb, *Arch. f. Entwicklungsmech.*, viii., 689, 1899.

of how necessary it is to form a clear conception of the notion of a ferment.

It is well known that a large portion of the excreted urea is *synthetically* formed in the liver from ammonium carbonate. Since now a series of *decomposing* functions of this organ can be attributed to enzymes, which are also active in aqueous extracts, the idea has occurred of determining whether the *synthesis of urea*, which can be demonstrated in the *still-living* organ after the blood has drained off, could not also be detected in aqueous extracts of the liver. SPITZER¹ draws an exact parallel between the processes of oxidation and the synthetic formation of urea, and is surprised that all attempts to produce urea from ammonium salts have been unsuccessful. We know that these attempts were *hopeless* from the first.

For the synthetic formation of urea is a *purely vital endothermic process*, which can be demonstrated in the surviving organs, but can never be attributed to the *action of a ferment*. A ferment can decompose and oxidise, but can never effect *syntheses*. The vital production of urea from ammonium salts is far rather to be placed on an analogy with the synthetic metabolism of *plants*.

There appears, however, to be a urea-producing ferment, although in quite another sense. RICHET,² for example, claims to have obtained from the liver an enzyme which could be precipitated by alcohol, and which formed urea by *decomposition* from more highly complex molecules. GOTTLIEB,³ too, found that there was an increase in the amount of urea on digesting an extract of liver under aseptic conditions. Theoretically this is quite possible, especially so, since we know that *arginine*, a tryptic decomposition-product of the proteids, is decomposed by simple hydrolysis, with the liberation of urea. Urea, indeed, has long been known as a hydrolytic decomposition product of proteids. It would thus be quite possible for there to be another source of urea in addition to its purely vital synthesis; that it is also formed by decomposition processes which are produced by *enzymes*. HOFMEISTER⁴ has shown that urea can also be formed by the combined decomposition and oxidation, particularly of amido-acids and oxy-acids, in the presence of ammonia.

Subsequently it was confirmed by CHASSEVANT and RICHET⁵

¹ Spitzer, *Pflüg. Arch.*, lxxi., 596, 1898.

² Richet, *C. R.*, cxviii., 1127. *C. R. Soc. Biol.*, 525, 1894.

³ Gottlieb, *Münch. med. Woch.*, 547, 1895.

⁴ Hofmeister, *A. f. exper. Path.*, xxxvii., 426, 1896.

⁵ Chassevant and Richet, *C. R. Soc. Biol.*, xlix., 743, 1897.

and by SCHWARZ¹ that *urea* increased in extracts of liver preserved under aseptic conditions; and that in this process *ammonium salts* and *proteids* and, according to Schwarz, also *oxamic acid* had no influence, but that *an addition of sodium urate*, and also, according to LOEWI,² of *glycocoll* and *leucine* caused the proportion of *urea* to rise considerably, whilst the *uric acid* decreased. It thus appears to effect a decomposition of *uric acid* into *urea*. According to LOEWI,² however, this substance is *not urea*, but *another* nitrogenous compound of which nothing further is known. JACOBY,³ who found the ferment in extracts of dogs' livers, appears rather to believe in a formation of *allantoin* from *uric acid*. This indeed occurs as a metabolic product of *uric acid*, and might easily be mistaken for *urea*. The decomposition of *uric acid* in the liver was also confirmed by ASCOLI.⁴

Loewi also obtained the ferment in a dry condition by precipitation with alcohol. The *existence* of a ferment of this kind is thus established, but it is still doubtful what it attacks and what product it yields.

The Glycolytic Ferment.—The question of the so-called "glycolytic" ferment of the blood and tissues is most intimately connected with that of the oxydases.

CL. BERNARD⁵ observed that the sugar in the blood disappeared fairly rapidly on standing, but yet attributed no special significance to this discovery. LÉPINE⁶ was the first to devote greater attention to this phenomenon, particularly with reference to the etiology of *diabetes mellitus*, the variety of diabetes in which sugar occurs in the urine. He came to the conclusion that the cause of the overloading of the blood with grape-sugar resulted from an insufficient assimilation of sugar; and that this again was due to a decrease in the glycolytic power of the blood, which he attributed to a *ferment*. The glycolytic function belongs to the *leucocytes*, whilst the serum is inactive.

Glycolysis is by no means a function of the activity of the cells, for the ferment can be extracted by means of a solution of common salt from the blood-corpuscles after separation in a centrifugal machine.

¹ Schwarz, *A. f. exper. Path.*, xli., 60, 1898.

² Loewi, *Z. physiol. Ch.*, xxv., 511, 1898.

³ Jacoby, *Virch. Arch.*, clvii., 235, 1899.

⁴ Ascoli, *Pflüg. Arch.*, lxxii., 340, 1898.

⁵ Cl. Bernard, *Vorlesg. üb. Diabetes*, 195, 1878; German translation by Posner.

⁶ Lépine. Detailed quotation from his own work in the *Wiener med. Presse*, No. 26 et seq., 1892.

The coagulated fibrin contains the ferment which, however, is attached not to the fibrin but to the entangled leucocytes. On washing the *coagulum* with water it passes into solution.

Lépine further assumes that this ferment can also become detached from the leucocytes *intra vitam*. Its formation is a function of the *pancreas*. When the pancreas is *stimulated*, e.g., by the removal of the Wirsungian duct, or severing of the nerves, the glycolysis is increased; on extirpation of the pancreas it is said to disappear. The pancreatic vein contains more ferment than the vein of the spleen. PÄL,¹ however, found that the amount of sugar in the pancreatic vein opposite the artery was *not* diminished.

The cessation of the normal function of the pancreas—i.e., of the glycolytic ferment—is regarded by Lépine as one of the causes of *Diabetes mellitus*, since in this disease a *diminution in the glycolytic capacity* of the blood runs parallel with the affection of the pancreas. This does not, however, apply to *phloridzin diabetes*.

In addition to this numerical diminution of the glycolysis, Lépine specially brings forward, in support of his view, an experiment, in which, in the case of a dog made diabetic by the extirpation of the pancreas, he was able to lower the excretion of sugar in the urine by injecting into the circulation *normal chyle*—i.e., containing ferment.

Whilst, then, the existence of a glycolytic ferment has been generally established, the views as to its mode of production, nature, and significance differ widely (HARLEY,² SANSONI,³ GAGLIO⁴).

SEEGEN⁵ and ARTHUS⁶ regard glycolysis as a *post-mortem* phenomenon. According to Arthus the ferment has its origin in the leucocytes, or as he cautiously expresses it:—"D'éléments figurés autres que les globules rouges." This receives support from an observation of HAHN,⁷ who was able to show the probability of an increase of the ferment in *hyperleucocytosis*.

* Arthus, in agreement with COLENBRANDER,⁸ brings the glycolytic function into close connection with the coagulation of the blood—i.e., the *fibrin*

¹ Päl, *Wiener klin. Woch.*, 4, 1891.

² Harley, *Journal of Physiol.*, xii., 391.

³ Sansoni, *Riforma medica*, 1891.

⁴ Gaglio, *Riforma medica*, 1891, quoted with the preceding reference by Minkowski, *Arch. f. Exp. Path.*, xxxi., 175, 1893.

⁵ Seegen, *Centralbl. f. Phys.*, v., Nos. 25, 26, 1891. *Wiener klin. Woch.*, 207, 1892.

⁶ Arthus, *Archives d. Phys.* [5], iii., 425, 1891; [5], iv., 337, 1892.

⁷ Hahn, *Berl. klin. Woch.*, 499, 1897.

⁸ Colenbrander, *Maly's Jb.*, 137, 1892.

ferment. The same substances which, through preserving the leucocytes, prevent the development of the fibrin ferment—*e.g.*, sodium fluoride and leech extract (COLENBRANDER)—also interfere with the glycolytic function. The influence of leech extract is confirmed by RYWOSCH.¹

Although LÉPINE (*loc. cit.*) has attempted to contest the relationship to the coagulation of the blood, and maintain the position of the glycolytic function as a distinct process, yet, in any case, the significance of his results for the pathogenesis of *Diabetes mellitus* has been very greatly reduced by the fact that other investigators (MINKOWSKI,² KRAUS,³ SPITZER⁴) were *not* able to confirm the statement that the glycolytic ferment was diminished in diabetes. Kraus, it is true, found the glycolytic ferment in the blood; he even proved that oxygen was absorbed in the process, and carbon dioxide produced; the glycolysis, however, according to him, is *not greater in normal than in diabetic blood*; it is only *relatively* greater on account of the smaller proportion of sugar.

Spitzer confirmed the statement that the glycolytic force of diabetic blood is the same as that of normal blood, and also showed that this oxidising force was not confined to the *cells of the blood*, but that it was common to *all* cells, from which it appeared that the glycolytic property of the blood was no longer to be regarded as a specific activity but as a manifestation of the *oxydases* mentioned above. SALKOWSKI,⁵ too, agrees with this view.

LÉPINE⁶ has asserted, in later publications, that the glycolytic ferment is not identical with the oxidising ferment, and has claimed that the former can be *artificially* obtained from malt-diastase by treatment with dilute (0·2 per cent.) sulphuric acid. These assertions, however, are contradicted by PADÉRI⁷ and by NASSE and FRAMM.⁸ JACOBY,⁹ again, maintains that there is a difference between the two ferments. He found that the glycolytic ferment was destroyed, even by a temperature of 58° C., whereas *salicylase* did not become completely inactive even at 75° C.

¹ Rywosch, *Centralbl. f. Phys.*, xi., 495, 1897.

² Minkowski, *Berl. klin. Woch.*, 5, 1892; *A. f. exp. Path.*, xxxi., 175, 1893.

³ Kraus, *Ztsch. f. klin. Med.*, xxi., 315.

⁴ Spitzer, *Berl. klin. Woch.*, 949, 1894. *Pflüg. Arch.*, lx., 303.

⁵ Salkowski, *Virch. A.*, cxlvii.

⁶ Lépine, *Comptes Rendus*, cxx., 139, 1895.

⁷ Padéri, *So. med. chir. de Pavia*, 1896. *Maly's Jb.*, 121, 1896.

⁸ Nasse and Framm, *Pflüg Arch.*, lxiii., 203, 1896.

⁹ Jacoby, *Virch. A.*, clvii., 235, 1899.

It behaves in every respect like the *enzymes* in general, is destroyed by boiling, combines with fresh fibrin, &c. According to Lépine, its optimum lies at about 45° C., and at 56° C. it is soon destroyed.¹ SEEGEN found that its action was favoured by the introduction of air. Blood-serum weakens its action, as does also, to a notable extent, foreign blood.¹ Seegen was unable to detect the formation of either *carbon dioxide* or *lactic acid*. Kraus found that there was an absorption of oxygen and production of carbon dioxide.

ACHARD and WEIL² also conclude that the glycolytic capacity is, *as a rule, diminished* in diabetes. They found that in diabetic patients the normal function of the organism to consume *glucose subcutaneously introduced* was weakened, so that sugar rapidly appeared in the urine; moreover, in the case of certain non-diabetic full-bodied individuals who were addicted to alcohol, it was possible to produce glycosuria in this way, so that Achard and Weil are inclined to speak of a "forme fruste" of diabetes.

On the other hand, only traces of galactose and fructose pass into the urine when these substances are subcutaneously injected in such cases.

My own experiments have, unfortunately, not yet led to any definite conclusion. I have endeavoured to throw light upon the question of what becomes of the sugar in the glycolysis in the blood. If the decomposition be regarded as *fermentative*, it is reasonable to suppose that there is either a formation of alcohol or of lactic acid. Both, indeed, are met with in the organism under certain conditions.

I was unable to detect alcohol, in any notable quantity at least, in blood into which sugar had artificially been introduced, and which had been left for forty-eight hours in an incubating chamber, with precautions to prevent putrefaction; I obtained, however, traces of a body which yielded iodoform, and which did not give Denigès' acetone reaction.

On the other hand, there appears, from the results of a series of experiments, to be a formation of lactic acid, so that, if these results are confirmed in the continuation of my work, the glycolytic ferment may be regarded as a *lactic-acid* producing enzyme (see *Lactic Acid Fermentation*).

In addition to the blood, the glycolytic ferment also occurs in various *organs*. Here, too, differences between it and the oxidising ferment appear. Thus, Jacoby (*loc. cit.*) found the *oxydase* in the liver of a diabetic patient, although the *glycolytic function* had disappeared. BLUMENTHAL³ found that the pancreas

¹ Hahn, *Berl. klin. Woch.*, 499, 1897.

² Achard and Weil, *C. R. Soc. Biol.*, 139, 986, 1898.

³ Blumenthal, *Z. f. physikal. u. diätet. Ther.*, 520, 1898.

had a strong glycolytic action, but only a slight oxidising power, whereas the *spleen* had exactly the contrary properties.

Blumenthal has obtained, by a method similar to that used by Buchner for the preparation of zymase (*vide supra*), extracts expressed at 75 to 100 atmospheres from the liver, &c.; and, in particular, from the *pancreas*, which have, he states, a glycolytic action, and also produce *carbon dioxide*.

Attempts to effect the cure of *diabetes* by means of such expressed glycolytic extracts have as yet yielded as little definite result as has feeding with *pancreas*, &c.¹ His results have recently been questioned by UMBER,² who found that the glycolytic capacity of the pancreas and pancreatic-venous blood was no greater than that possessed by the blood in general. He ascribed the evolution of gas from the expressed extracts to the action of bacteria.

PIERALLINI³ could not detect as great a glycolytic capacity in the human pancreas (from cadaveric material) as Blumenthal found in the fresh organs.

The occurrence of a glycolytic ferment in urine is very questionable.

Jecorin.—Since the measurement of the amount of sugar in the blood is based on its reducing capacity, all methods of estimating sugar in the blood suffer from the drawback that, for the most part, the sugar in the blood does not occur in the *free* state, but in combination with *lecithin*. From this combination it is only liberated by decomposition, whilst this compound by itself also causes some reduction.

This compound, which is soluble in ether, was first found by DRECHSEL⁴ in the *liver*, and named *jecorin* by him. He regarded it as a compound of *lecithin* with a sugar. This was confirmed by MANASSE,⁵ and the sugar recognised as *glucose*. *Jecorin* was found in the blood by JACOBSEN⁶ and HENRIQUES.⁷ BING⁸ then succeeded in proving that sugar which is introduced into the blood also *enters into combination* to form *jecorin*, and that a substance closely resembling *jecorin* can also be obtained from pure *lecithin* and grape sugar.

The Oxydases of Plants.—It was observed by SCHÖNBEIN⁹ that the cells of plants contained substances with a catalytic

¹ For the bibliography, see Blumenthal, *loc. cit.*

² UMBER, *Z. klin. Med.*, xxxix., 12, 1900.

³ Pierallini, *Z. klin. Med.*, xxxix., 26, 1900.

⁴ Drechsel, *J. f. pr. Ch.*, N.S., xxxiii., 425, 1886.

⁵ Manasse, *Z. physiol. Ch.*, xx., 478, 1895.

⁶ Jacobsen, *Centralbl. f. Phys.*, vi., 369, 1892.

⁷ Henriques, *Z. physiol. Ch.*, xxiii., 244, 1897.

⁸ Bing, *Centralbl. f. Physiol.*, xii., 210, 1898.

⁹ Schönbein and others, *Z. f. Biol.*, 1888. A survey of the whole of Schönbein's researches on this subject is given by Schaer, *Z. f. Biol.*, xxxvii., 320, 1899.

action similar to those in animal cells, which were recognised by their action upon *hydrogen peroxide*, and by the spontaneous alterations in colour—*e.g.*, of *fungi*. The spontaneous oxidation of natural vegetable chromogens was then described by PFEFFER.¹ STRUVE² found that pyrogallol was oxidised to purpurogallin when brought into contact with gum arabic; VAN DEN BROEK³ observed that the extracts from many plants turned guaiacum tincture blue, as was also found by SCHAEER⁴ to be the case with *Phytolacca decandra*, malt infusion, &c. POHL⁵ was able to obtain the *indophenol* reaction with vegetable extracts—*e.g.*, of pine-needles—but could not effect the *oxidation of formaldehyde* by their means.

Then BERTRAND⁶ published in numerous communications the results of his researches on an oxidising ferment, *laccase*, which he had first obtained from the Tonkin lac-tree, *Rhus vernicifera*. This ferment, which had already been briefly described by YOSHIDA,⁷ causes the oxidation of the yellow sap of the bark into a beautiful deep-black lac. Bertrand also discovered it in many *phanerogams* and *fungi*, and also in *gum arabic*. *Laccase* consists for the most part of carbohydrates, which in the decomposition yield galactose and arabinose, and of mineral constituents rich in manganese. It is stated to be *devoid of nitrogen*. It is specially characterised by the fact that it oxidises multi-valent *phenols*—such as *pyrogallol*, *hydroquinone*, &c., but leaves the simple phenols intact. Moreover, meta-phenols—such as *phloroglucinol* and *metamidophenol* remain unaltered, whilst para-phenols and, in particular, *hydroquinone* are readily attacked. His observations have been confirmed by other French investigators. TOLOMEI⁸ found a ferment resembling laccase in wine, and attributed to it a share in the production of the *bouquet* (*vide infra* "*Oinoxydase*").

BOURQUELOT⁹ found that in the successive action of *emulsin* and a vegetable *oxydase* upon *salicin* salicylic aldehyde was formed from the salicylic alcohol first produced, and concluded that a similar process might very

¹ Pfeffer, *Ber. d. d. bot. Ges.*, iii., 82, 1889.

² Struve, *Ann. d. Chem. u. Pharmac.*, clxiii., 160. Quoted from Bertrand, *loc. cit.*

³ van den Broek, *Jahresb. d. Ch. v. Liebig u. Kopp*, 455, 1849-50.

⁴ Schaer, *Apothekerztg.*, 749, 1894.

⁵ Pohl, *A. f. exp. Path.*, xxxviii., 65.

⁶ Bertrand, *Comptes Rendus*, cxviii., 1215; cxx., 266; cxxi., 166, 783; cxxii., 1132. *Archiv. d. Physiol.*, 23, 1896.

⁷ Yoshida, *Journ. Chem. Soc.*, xliii., 472, 1883.

⁸ Tolomei, *Maly's Jb.*, 913, 1896.

⁹ Bourquelot, *C. R. Soc. Biol.*, xlviii., 516, 1896.

well play a part in the plants themselves—e.g., *Spiræa ulmaria*, in which salicylic aldehyde occurs.

BERTRAND¹ claims to have found another oxydase, *tyrosinase*, which has a specific action upon *tyrosin*, in the juice of the beet, in the *dahlia*, and in certain *fungi*, notably *Russula*. It is said to be an effective cause of the spontaneous dark coloration of beet juice. According to Bertrand it is distinct from *laccase*, with which it is associated. The ferment is very unstable, being destroyed by heating to 55° C. and by alcohol.

HARLAY² employs the *tyrosinase* of *Russula delica* for the detection of *tyrosin* in the mixed products of digestion, and claims to be able by its means to distinguish between peptic and tryptic digestion. He also observed the typical brown coloration of *tyrosin* in papain digestions.

Laccase has no action upon *tyrosin*. In his numerous researches on the enzymes of *fungi*, BOURQUELOT³ has described, in addition to proteolytic ferments, oxidising ferments, which oxidise all phenols.⁴ He also found *tyrosinase*.⁵ Oxydases were also discovered by him in different gums.⁶

REY-PAILHADE⁷ found in parts of plants that characteristic ferment found in animal organs, which gives the *indophenol reaction*, which *laccase* does not, but differs from the animal ferment in being soluble in water and dilute alcohol.

CORNU⁸ has found in almost all the organs of the *vine*, oxydases which are destroyed by absolute alcohol.

The phosphorescence of animals and plants is attributed by DUBOIS⁹ to an oxidising ferment, to which he has given the poetic name of *luciferase*.

The fermentation of tobacco leaves, which has hitherto been regarded as the work of bacteria, is also stated by LOEW¹⁰ to be due to an oxydase.

Another oxidising ferment is said to cause the "browning" or sudden colour-change of wine, a spontaneously-occurring discoloration. It is said to resemble *laccase*, and has been named *oinoxydase*. It can be obtained from the wine by precipitation with alcohol, in combination with a body of the nature of a gum. It is also stated to play a part in the *maturing* of

¹ Bertrand, *Comptes Rendus*, cxxii., 1215; cxxiii., 463. *Bull. Soc. Chim.*, 793, 1896.

² Harlay, *Chem. Centralbl.*, 1899, ii., 850; 1900, i., 676.

³ Bourquelot, *Journ. d. Pharm. et Chim.* [6], iv., 145, 241, 440; v., 465; vi., 426. *C. R. Soc. Biol.*, xlviii., 811, 825, 893, 896, 1896. Bourquelot and Bertrand, *Bull. Soc. Myc.*, xii., 18, 27. Reprint.

⁴ Bourquelot, *Comptes Rendus*, cxxiii., 315, 423.

⁵ Bourquelot, *Bull. Soc. Mycol.*, xiii., 65. Reprint.

⁶ Bourquelot, *C. R. Soc. Biol.*, xlix., 25, 1897.

⁷ Rey-Pailhade, *C. R. Soc. Biol.*, xlviii., 479, 1896.

⁸ Cornu, *Journ. d. Pharm. et Chim.* [6], x., 342, 1899.

⁹ Dubois, *Comptes Rendus*, cxxiii., 653, 1896.

¹⁰ Loew, *C. f. Bakteriolog.* [II.], vi., 109, 1900. Quoted by *Chem. Ztg.* (March), 1900.

wine. It is destroyed on pasteurising the wine at 60° C., and also by sulphurous acid; this is said to partially account for the beneficial effect of sulphuring the casks (GOUIRAND,¹ MARTINAND,² LABORDE,³ CAZENEUVE,⁴ BOUFFARD,⁵ and others). Laborde identifies its activity with that of the fungus *Botrytis cinerea*, which, however, is disputed by Cazeneuve. *Aspergillus*, &c., produce no *oinoxydase*. BRISSEMORET and JEANNE⁶ discovered an oxydase in *digitalis*. LINDET⁷ attributes the darkening of apple-juice to an oxydase which oxidises the tannin.

In considering this wholesale production of *oxydases*, &c., with those which the French biological chemists presented to us some years ago, we have the uneasy, dispiriting feeling that a large proportion of these *enzymes* would not stand the test of a close investigation, particularly since not one of them has been prepared in an approximately pure condition. The only German chemist who, to my knowledge, has studied *laccase* more closely is greatly inclined to attribute their "fermentative activity" to the long-known catalytic action of the manganese salts, of which they contain a large proportion (RUFF⁸).

This opinion appears to be gradually gaining ground, even among the French. At least BERTRAND⁹ has thought the catalytic action of the manganese salts in *laccase* worth a fuller investigation, and ascribes great importance to them. He does not, indeed, yet abandon the specific activity of *laccase*, but considers that the manganese salts can only be credited with a *subsidiary* force, and therefore terms them *co-ferments*. The *laccase* from lucernes contains very little manganese, but also has a very weak action. He assumes that manganous oxide acts as the *conveyor of oxygen*, being alternately oxidised to manganese dioxide and giving up its oxygen again. The ferment itself is considered to be a salt-like compound of manganese oxide with a proteid nucleus, the former being the conveyor of the activity. He thus formulates speculations similar to those of Spitzer on his nucleo-proteids (*vide* p. 287).

The theory of the *oxydases* also receives a severe blow by the discovery of NASSE and FRAMM¹⁰ that the blue coloration of

¹ Gouirand, *Comptes Rendus*, cxx., 887, 1895.

² Martinand, *Comptes Rendus*, cxx., 1426.

³ Laborde, *Comptes Rendus*, cxxiii., 1074, 1896.

⁴ Cazeneuve, *Comptes Rendus*, cxxiv., 406, 781, 1897.

⁵ Bouffard, *Comptes Rendus*, cxxix., 706.

⁶ Brissemoret and Jeanne, *Journ. Pharm. et Chim.* [6], viii., 481; *Chem. Centralb.*, i., 133, 1899.

⁷ Lindet, *Comptes Rendus*, cxx., 370, 1895.

⁸ Ruff. *Private communication*.

⁹ Bertrand, *Comptes Rendus*, cxxiv., 1032, 1355, 1897. Cf. Denigès, *Comptes Rendus*, cxxx., 32, 1900.

¹⁰ Nasse and Framm, *Pflüg. A.*, lxiii., 203, 1896.

guaiacum by the plant juices also occurs *in the absence of oxygen*; they are much more inclined to assume the existence of *hydroxylising ferments*.

Be that as it may, the "ferments" which produce the blue coloration of guaiacum are still the most reliable. They occur, as was briefly mentioned above, in very many plants and parts of plants. As regards their activity, they can be classified into *direct oxydases* and *indirect oxydases*, which only turn guaiacum tincture blue through the medium of hydrogen peroxide, and which were first closely studied by PFEFFER.¹

RACIBORSKI² has observed this blue coloration with *hydrogen peroxide* and *guaiacum* in very many plants, and notably in the *leptoxylem*, and attributes it to a special substance—*leptomin*. GRÜSS,³ later on, subjected the whole subject to a close investigation. He also found the "indirect" oxydases in the *phloëm*, and in resting trees, in the *newest wood of all*, in addition to the *leptoxylem*; but not in the *pith*, in the *xylem*, or in the *bark*. After the winter's rest the *medullary rays* also began to show the reaction.

On the other hand, he found *direct oxydases* specially in the walls of the vessels.

I cannot, of course, go into all the anatomical particulars of Grüss's work here, though I must still mention the very far-reaching theoretical conclusions which he draws from his results.

Thus he propounds the theory that these oxidation reactions are very intimately connected with *diastase*. Although he quotes Jacobsen's proof that a *catalytic action* can be attributed to diastase (see p. 45), he yet considers that he is justified, on the strength of his own experiments, in concluding that it is an essential characteristic of the diastase *itself* to bring about these catalytic actions. In this sense he regards the manifestation of certain catalytic reactions as very intimately connected and possibly identical with that of *diastase*, particularly the *translocation diastase* of Brown and Morris (see p. 170). He thus arrives at a classification of the oxidising ferments of plants into three groups:—

1. The α -oxydases are direct oxydases; they occur in different parts of the plants, can be extracted with glycerin, and are destroyed by alcohol.

2. The β -oxydases are only active in the presence of hydrogen peroxide, and resist the influence of oxygen. To this group

¹ Pfeffer, *Ber. d. d. botan. Ges.*, iii., 82, 1889.

² Raciborski, *Ber. d. d. botan. Ges.*, xvi., 119, 1898.

³ Grüss, *ibid.*

belongs Raciborski's *leptomin*. They thus correspond to the *indirect oxydases*.

3. The γ -oxydases. They manifest themselves under special circumstances, *e.g.*, after a hole has been bored into a potato and the wound has been allowed to heal, they are found *only* in the periderm cells of this wound. They have a *strong hydrolytic action*, but a *weak catalytic* one. And to this group, according to Grüss, belongs diastase, specially *translocation diastase*, though also *secretion diastase* and *cytase*; on the other hand, the diastase of *penicillium* does *not* possess oxidising properties.¹

One can hardly accept this view, although it is difficult, especially for one who is not a botanist, to refute it experimentally. It remains to be seen whether it will not be opposed by experts in this subject.² But we can hardly do otherwise than conclude that, although under certain conditions, diastase is possibly constantly accompanied by such catalytic substances, so that this reaction can be used with advantage for the detection of the ferment in the organs of plants; yet there is still no sufficient ground for believing in the actual *identity* of diastase with such substances.

¹ Grüss, *Festschr. f. Schwendener*, 184, 1899 (Berlin).

² This has since been done by Raciborski. See *Flora*, 1900.

CHAPTER XXIV.

OXIDISING FERMENTATIONS.

WHILST in the oxidising fermentations of which we have yet spoken, we have been dealing with *enzymes*, which effect the transference of oxygen without the co-operation of living cells, there is yet a series of processes in which oxidation occurs in such intimate connection with the living cells that it has been impossible, until recently at least, to isolate the active ferments from them. Yet here, too, we have to deal with exothermic processes of a specific nature, so that we have some justification for regarding them as *fermentative processes*.

The main representative of this class of fermentations is

The Acetic Fermentation.—The fact that dilute alcohol becomes acid on standing has been known and applied to practical purpose from the earliest times. The chemical process which occurred was naturally not known until the beginning of modern chemical investigation. The first to observe the absorption of air—*i.e.*, oxygen—in this acetification of wine was ROZIER¹ towards the end of the 18th century. The equation of the process was then established mainly by DOBEREINER.²

At a later period a contest raged between LIEBIG³ and PASTEUR⁴ over the cause of the acetic fermentation on the same lines as that of which we have given an outline in discussing alcoholic fermentation. Here, too, Liebig's view of a fermentation produced by the spontaneous decomposition of albuminoid substances was opposed by the vital explanation of Pasteur's School, and here, too, the victory inclined to the supporters of the vitalistic theory. The connection of the acetic acid fermentation with a series of lower organisms was irrefutably proved.⁵ Before going into this question further, we must first consider the *chemistry of the reaction*. It proceeds very simply in accordance with the equation—

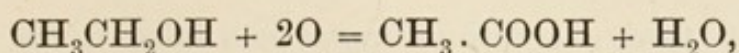
¹ Quoted by A. Mayer, *Gährungschemie*, 170.

² Dobereiner, *Schweigger's Journ. f. Chemie.*, viii., 321.

³ Liebig, see, *inter alia*, *Journ. f. pr. Ch.*, N. S., i., 35, 312.

⁴ Pasteur, see in particular *Etudes sur le Vinaigre*, Paris, 1868.

⁵ For the history of the acetic fermentation see Lafar, *C. f. Bakt.*, xiii., 684, 1893.



in which we must conclude that there is an intermediate formation of *acetaldehyde*, $\text{CH}_3\cdot\text{CHO}$.

The fermentative reaction strictly follows the course here represented, for the fact that small quantities of aldehyde can invariably be detected is not due to a secondary simultaneous process, but only represents the momentary stage in the course of the reaction, in which a production and further oxidation of the aldehyde is continually taking place.

The conditions under which the production of vinegar proceeds belong, in the main, to the consideration of the influence of micro-organisms in general; it only takes place in dilute solutions of alcohol, thriving best at 25° to 30° C., is very slow below 10° C. and above 35° C., and completely stops at a temperature but little higher than this.¹

The Biology of the Acetic Fermentation.—As the liquids become sour a pellicle is formed upon them, the so-called *mother-of-vinegar*, which Liebig regarded as the ferment, which, in its decomposition, effected the transference of oxygen.

This pellicle, however, was discovered, first by KÜTZING² and then by THOMSON,³ to be composed of living vegetable cells, which were subsequently described, notably by PASTEUR,⁴ under the name of *Mycoderma aceti*, and regarded as the effective cause of the acetic fermentation. Since, however, the name *mycoderma* would indicate a relationship with the *budding-fungi*, which form similar pellicles upon alcoholic liquids without inducing acetic fermentation, the organisms which cause that fermentation have been grouped, in accordance with Zopf's proposal, under the generic name *Bacterium*, which indicates their true position among the *fission-fungi*.

A whole series of such acetic bacteria are now known. Besides the *B. aceti*, there are also recognised *B. Pasteurianum*, *B. Kuetzingianum* (HANSEN⁵), *B. oxidans*, *B. acetosum*, *B. acetigenum*, *Termobacterium aceti*, and many others.⁶

¹ By acclimatisation to a different method of manufacture, acetic bacteria in England thrive best at a much higher temperature— 40° to 44° C.—Translator.

² Kützing, *J. pr. Ch.*, xi., 390.

³ Thomson, *Ann. Chem. Pharm.*, lxxxiii., 89, 1852.

⁴ Pasteur, see in particular *Études sur le Vinaigre*, Paris, 1896.

⁵ Hansen, *Untersuch. a. d. Technik. d. Gährungsgewerbes*, 1895.

⁶ Cf., *inter alios*, Wermisheff, *Ann. Past.*, 213, 1893; Henneberg, *C. f. Bakt.*, [2], iv., 14, 71, 1898; Hoyer, *ibid.*, 867; *Chem. Centralbl.*, i., 854, 1899.

The fact that a *budding-fungus* can also cause acetic fermentation has been recorded by LAFAR.¹ This, however, is not the case with the *Saccharomyces mycoderma* Reess, which does *not* induce acetic fermentation, but consumes the sugar directly.

The spores of these micro-organisms are of universal occurrence in the atmosphere, so that alcoholic liquids exposed to the air are soon infected by them. If, on the other hand, the air is excluded, the liquids remain sterile, and no acid fermentation ensues. The fact that the presence of these bacteria is absolutely necessary for the acetic fermentation has been demonstrated in the same manner as in alcoholic fermentation, so that we need not go into this more closely here. Their vital conditions, too, closely resemble those of other micro-organisms. They are naturally *aërophile* of necessity, thrive in all nutrient liquids, and are able, like the yeasts, to obtain their necessary nitrogen from ammonium salts.

They are killed at about 60° C., whilst their fermentative activity is extinguished at a somewhat lower temperature. At temperatures below 12° to 15° C. their activity is weakened; moreover, they are acted upon in a perfectly analogous manner to yeasts by protoplasm-poisons, though they exhibit a greater sensitiveness to the action of *sulphurous acid*, so that wines can be successfully protected against their action by "sulphuring." It has been shown by GIUNTI² that their development is greatly checked by *direct sunlight*. TOLOMEI³ found that *currents of electricity* prevented the acetic fermentation, though only during their actual passage. Alcohol in the proportion of more than 10 per cent. is fatal to them. One characteristic they naturally possess is that, in addition to their great sensitiveness to the action of *alkalies* (HENNEBERG⁴), they offer great resistance to *acetic acid*. They appear to first attain their full vital activity when the proportion of acid reaches 2 per cent., but they are also unaffected by much greater degrees of concentration. We have here a very remarkable example of extreme adaptation to the environment, since bacteria are usually highly sensitive to the influence of acids. They are, however, very sensitive to *hydrochloric acid* (COHN⁵). They are also capable of effecting the fermentation of *glucose*, and some, also, that of *arabinose*, *mannite*,

¹ Lafar, *C. f. Bakter.*, xiii., 1864, 1893.

² Giunti, *Maly's Jb.*, xx., 439, 1890.

³ Tolomei, *Koch's Jahrb. Gährsorg.*, 139, 1890.

⁴ Henneberg, *loc. cit.*

⁵ Cohn, *Z. physiol. Ch.*, xiv., 75, 1890.

erythrite, &c. In like manner they can also oxidise *propyl alcohol* (HENNEBERG¹).

Liebig found a striking phenomenon in support of his chemical theory in the fact that the same oxidation of alcohol to acetic acid could also be effected by means of *platinum black*—i.e., of oxygen rendered active without the aid of living cells. A. MAYER and KNIEREM,² however, have shown that the conditions of these reactions are so totally different that only their *end-results* can be compared, just as in the case of hydrolytic fermentations the same decomposition-products can also be obtained by the action of dilute acids, and just as alcohol can, of course, be converted into acetic acid by other purely chemical means—e.g., *chromic acid*, &c. Thus, the oxidation of alcohol by platinum black takes place with alcohol of every strength, and in like manner with its vapour, whilst the acetic fermentation cannot withstand more than 10 per cent. of alcohol. The former also increases as the temperature rises, whereas the fermentation eventually completely ceases.

We must, nevertheless, call attention to the fact that, in the case of the acetic fermentation, there is *absolutely no observed fact* from which we can infer that the hypothetical fermentation is distinct from the purely vital process, of which, in the case of alcoholic fermentation by yeast, certain indications could be found, apart from Buchner's convincing experiments.

Only the dynamic position which we have taken up justifies us in classifying with the fermentative processes this process, which, being exothermic, fulfils our conditions. Possibly, however, the day is not far distant when the *oxydase* of the acetic bacteria will also be isolated from the living cells.

In addition to this acetic fermentation, which represents the most important oxidising fermentation, there are also some allied processes brought about by bacteria, which we will also briefly mention as belonging to the oxidising fermentations.

ZOPF³ describes a fermentation of sugars—e.g., of *d-glucose*, galactose, mannite, &c.—into *oxalic acid*, which is caused by a true endospore forming species of *Saccharomyces*, *S. Hansenii*, which was discovered in cotton-seed meal. It produces no alcohol.

¹ Henneberg, *loc. cit.*

² A. Mayer and Knierem, *Landw. Versuchsstat.*, xvi., 305. Quoted by A. Mayer, *loc. cit.*

³ Zopf, *Ber. d. d. botan. Ges.*, 94, 1889.

According to WEHMER,¹ *citric acid* is produced in abundance from sugars by two specific *Hyphomycetes*—*Citromyces Pfefferianus* and *C. glaber*; this process, too, can be regarded as an oxidising fermentation.

BERTRAND² describes an oxidising fermentation, the product of which is a ketohexose—*Sorbose*.

Sorbose had already been found in the juice of mountain-ash berries which, when fresh, contain the hexavalent alcohol *sorbite*, but it was not found invariably, and the conditions of its production were unknown.

Bertrand proved that this formation of *sorbose* was connected with the development of a micro-organism, which he considered as probably identical with *Bact. xylinum*,³ and which gain admittance into the material through the agency of a small red fly, *Drosophila funebris*. The best culture-medium for this bacterium is a mixture of wine and vinegar. It has the power of oxidising *sorbite* to *sorbose*, and mannite to *fructose* (VINCET and DELACHANEL⁴); it also oxidises all polyatomic alcohols, such as *erythrite*, *arabite*, &c.; also *glycerin* (to crystalline *dioxyacetone*), and *xylose* to *xyloic acid* (BERTRAND⁵). A bacterial ferment which oxidised glucose to *gluconic acid* was found by BOUTROUX in *Micrococcus oblongus*; and another which further oxidised the gluconic acid, first produced in the form of its calcium salt, into *oxygluconic acid*.⁶

There can be no doubt but that there is still many another process among the decompositions brought about by the lower organisms which could be justifiably described as an oxidising fermentation; I have, however, only selected these few because their course is relatively simple and uniform, whilst others are too closely bound up with other bio-chemical processes. In any case, those cited here fully answer the dynamic requirement, and may, therefore, well be grouped with the fermentative processes.

¹ Wehmer, *Sitzb. d. Berl. Acad. Math. Phys. Cl.*, 519, 1893.

² Bertrand, *Comptes Rendus*, cxxii., 900, 1896.

³ This has been confirmed by Emmerling, *vide Ber. d. d. chem. Ges.*, xxxii., 541, 1899.

⁴ Vincet and Delachanel, *Comptes Rendus*, cxxv., 716, 1897.

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¹ It is obvious that from the nature of the publications on our subject a somewhat arbitrary selection is necessary if continued repetition is to be avoided. The references marked with an asterisk (*) have been compiled at second-hand.

² I only had access to this after my manuscript was practically finished.

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XIV. Ferments of Disaccharides.

I = Invertase.

M = Maltase.

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 769. Bau, *Z. f. Spir. Ind.*, 232, 1899 (Trehalase).
 770. Bauer, *Chem. Zeit.*, 1895, 1873 (Melibiase).
 771. Berthelot, *C. R.*, li., 980, 1860 (I).
 772. Beyerinck, *C. f. Bakt.*, vi., 44, 1889 (Lactase).
 773. Bourquelot, *J. Anat. et Phys.*, xxii., 162, 1886.
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 787. Gley and Bourquelot, *C. R. Soc. Biol.*, 247, 1895.
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804. Shore and Tebb, *J. of Physiol.*, xiii., 19 (M).
 805. O'Sullivan, *J. Chem. Soc.*, lxi., 593, 1892 (I).
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XV. Ferments which Decompose Glucosides.

E = Emulsin. M = Myrosin.

808. Berg, *Bull. Soc. Chim.* [3], xvii., 85, 1897 (Elaterase).
 809. Beyerinck, *C. f. Bakt.* [II.], v., 425, 1899 (Gaultherase).
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 811. *Bougarel, *Sur l'Amygdaline*, Thèse, Paris, 1877 (E).
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 816-17. Bréaudat, *Bull. Soc. Biol.* [10], v., 1031, 1898. *C. R.*, cxxvii., 769, 1898.
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 819. Bussy, *Lieb. Ann.*, xxxiv., 223, 1840 (M).
 820. Fauré, *J. d. Pharm.*, xvii., 279 (M).
 821. E. Fischer, *Ber. Chem.*, xxviii., 1508, 1895 (E).
 822. Gérard, *C. R.*, cxxiv., 370, 1895 (E).
 823-24. Guignard, *J. d. Bot.*, iv., 3, 19, 385, 1890 (E, M).
 825. Guignard, *J. Pharm. Chim.* [5], xxi., 233, 1890.
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 829. Hubatka, *Lieb. Ann.*, xlvii., 157, 1843 (M).
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 833. Kawalier, *J. pr. Ch.*, lviii., 193, 1853 (E).
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 835. Liebig and Wöhler, *Lieb. Ann.*, xxii., 1, 1837 (E).
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 839. *Lütz, *Bull. Soc. Bot. d. France*, xlv., 26, 263, 1897 (E).
 840. Ortloff, *Arch. d. Pharm.*, xlviii., 12, 1846 (E).
 841. Piria, *Ann. Chim. Phys.* [3], xiv., 257, 1845 (E).
 842. Pless, *Lieb. Ann.*, lviii., 36, 1846 (M).
 843. Poleck, *Pharm. Ztg.*, 314, 1891 (E).
 844. Portes, *J. Pharm. Chim.*, xxvi., 410, 1877 (E).
 845. *Procter, *Amer. J. of Pharm.*, xv., 241 (Gaultherase).
 846. Puriewitsch, *Ber. bot. Ges.*, xvi., 1898. *Bull. Soc. Biol.* [10], iv., 686, 1897.
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 853. Schneegans, *J. d. Pharm. v. Els.-Löthr.*, 17, 1896 (Gaultherase).
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 864. Will and Körner, *Lieb. Ann.*, cxxv., 257, 1863 (M).
 865. Will and Laubenheimer, *ibid.*, cxcix., 162, 1879 (M).

XVI.—Other Hydrolytic Ferments.

L = Lipase. U = Urea-fermentation.

866. Achard and Clerc, *C. R.*, cxxix., 281, 1899 (L).
 867. Boerhave, *Elem. Chim.*, London, 1732 (U).
 868. Brodmeier, *C. f. Bakt.*, xviii., 380, 1895 (U).
 869. *Cambier, *Ann. d. Microgr.*, 1893 (U).
 870. Cazeneuve and Livon, *C. R.*, lxxxv., 571 (U).
 871. Connstein, *Med. Woch.*, No. 15, 1900 (L).
 872-73. Connstein and Michaelis, *Pflüg. Arch.*, lxx., 473; lxix., 76 (L).
 874. H. Fischer, *Berl. klin. Woch.*, 18, 1864 (U).
 875. Fleury, *Ann. Chim. Phys.* [4], iv., 38, 1865 (L).
 876. Fourcroy and Vauquelin, *Ann. d. Chim.*, xxxi., 48; xxxii., 80, 113 (U).
 877. Gérard, *C. R. Soc. Biol.*, xlviii., 516, 1896 (U).
 878. Green, *Proc. Roy. Soc.*, xlviii., 370, 1890 (L).
 879. Grützner, *Pflüg. Arch.*, xii., 302 (L).
 880. *Guiard, *Transform. Ammon. des Urines*, Thèse, Paris, 1883.
 881. Hankel, *Schmidt's Jb.*, iii., 1 (U).
 882. Hanriot, *C. R.*, cxxiii., 753; cxxiv., 778, 1896-97 (L).
 883. Hanriot, *C. R. Soc. Biol.*, xlviii., 925, 1896 (L).
 884-85. Hanriot and Camus, *C. R.*, cxxiii., 831; cxxiv., 235 (L).
 886. Heritsch, *Centr. med. Wiss.*, 449, 1875 (L).
 887. Hoppe-Seyler, *Pflüg. Arch.*, xii., 1 (Calcium formate).
 888. v. Jaksch, *Z. ph. Ch.*, v., 395 (U).
 888a. Kastle and Loevenhart, *Amer. Chem. J.*, xxiv., 491, 1900 (L).
 889. Klüg, *Pflüg. Arch.*, lxx., 329 (L).
 890. Knauthe, *Du Bois Arch. f. Phys.*, 149, 1898 (L).
 891. Ladureau, *C. R.*, xcix., 877, 1884 (U).
 892. Lea, *J. of Phys.*, vi., 136 (U).
 893. Leone and Sestini, *Landw. Versuchsstat.*, xxxviii., 157, 1891 (U).
 894-95. Leube, *Z. klin. Med.*, iii., 233, 1881. *Virch. Arch.*, c., 540 (U).
 896. Lumia, *Maly's Jb.*, 724, 1899 (L).
 897-99. Miquel, *Bull. Soc. Chim.*, xxix., 387; xxxi., 391; xxxii., 126, 1878 et seq. (U).

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 909-10. Musculus, *C. R.*, lxxviii., 132. *Pflüg. Arch.*, xii., 214 (U).
 911. Ogata, *Du Bois Arch. f. Phys.*, 515, 1881 (L).
 912. Pasteur, *C. R.*, l., 849, 1860 (U).
 913. Pasteur and Joubert, *ib.*, lxxxiii., 5, 1876 (U).
 914. Pelouze, *Ann. Chim. Phys.* [3], xlv., 319 (L).
 915. Proust, *Ann. d. Chim.* [2], xiv., 257 (U).
 916. Prout, *Ann. of Philosoph.*, xi., 352, 1818 (U).
 917. Popoff, *Pflüg. Arch.*, x., 142 (Calcium formate).
 918-19. Sachs, *Botan. Ztg.*, 178, 1859; 242, 1862 (L).
 920. Shearman, *Schmidt's Jahrb.*, lv., 276 (U).
 921-22. Sigmund, *Monatsh. f. Chemie*, xi., 272, 1890; xiii., 1892 (L).
 923-24. van Tieghem, *C. R.*, lii., 210; lviii., 210 (U).

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 927. Boutron-Charlard and Frémy, *Ann. Chim. Phys.* [3], ii., 257, 1841.
 928. Boutroux, *C. R.*, lxxxvi., 615, 1878.
 929. *Braconnot, *Ann. Chim. Phys.*, xxxvi., 116.
 930. Chassevant and Richet, *C. R.*, cxvii., 673, 1893.
 931. Cohn, *Z. phys. Ch.*, xiv., 25, 1890.
 932. Du Bois-Reymond, *Sitzb. Berl. Acad.*, 288, 1859.
 933. Frankland and MacGregor, *J. Chem. Soc.*, lxiii., 1028, 1893.
 934. Gay-Lussac and Pelouze, *Ann. Chim. Phys.*, lii., 410, 1833.
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 939. Kuprianow, *Arch. f. Hyg.*, xix., 282, 1893.
 940. Leichmann, *C. f. Bakt.*, xvi., 826.
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 944-46. Pasteur, *C. R.*, xlv., 913; xlvii., 224; xlviii., 337, 1857-8.
 947-48. Péré, *Ann. Inst. Past.*, vi., 737, 1892-3.
 949. Richet, *C. R.*, cxiv., 1494, 1892.
 950. Schardinger, *Monatsh. f. Chem.*, xi., 545, 1890.
 951. Tate, *J. Chem. Soc.*, lxiii., 1263, 1893.
 952. Timpe, *Arch. f. Hyg.*, xviii., 1, 1893.

XVIII.—Alcoholic Fermentation.

Z = Zymase (also other occurrence of Alcohol and CO₂ in plants).

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 954. Adametz, *C. f. Bakt.*, v., 116, 1889.
 955. *Adrian, *Bull. gén. Thér.*, 156, 1900 (Z).
 956. Albert and Buchner, *Ber. Chem.*, xxxiii., 266, 971, 1900 (Z).

957. Baeyer, *Ber. Chem.*, iii., 73, 1870.
958. Barfoed, *J. pr. Ch.* [2], vi., 334, 1872.
959. Bert, *C. R.*, lxxx., 1579.
- 960-61. Berthelot, *C. R.*, lix., 904, 1864; cxxviii., 1366, 1899 (Z).
962. Beyerinck, *C. f. Bakt.*, xvi., 49, 1894.
963. Boehm, *Sitzb. Wien. Acad.*, lxvii. [1], 211, 1873 (Z).
964. Bouchardat, *C. R.*, xviii., 1120, 1844.
- 965-66. Bourquelot, *C. R. Soc. Biol.*, 191, 221, 356, 1885.
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- 968-70. Brefeld, *Ber. Chem.*, vii., 281, 1066; viii., 421, 1875.
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984. Cagniard-Latour, *Ann. Chim. Phys.* [2], lxviii., 206, 1836.
- 985-86. Cahours, *C. R.*, lviii., 495, 635, 1864 (Z).
987. Claudon and Morin, *C. R.*, civ., 1109, 1887.
988. Cochin, *C. R.*, xcvi., 852, 1883.
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990. *Delbrück, *Woch. f. Brauerei*, 1895.
991. Devaux, *C. R.*, cxxviii., 1346, 1899 (Z).
992. Dienert, *C. R.* cxxviii., 569.
993. Dienert, *Ann. Inst. Past.*, xiv., 139, 1900.
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1001. Dumont, *Trommsdorff's Journ. f. Pharm.*, iii. [2], 563, 1819 (Z).
- 1002-6. Effront, *Bull. Soc. Chim.* [3], v., 148, 476, 731; vi., 786; ix., 151, 1893.
1007. Effront, *C. R.*, cxix., 92, 169, 1894.
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- 1011-12. Foth, *Z. ges. Brauw.*, 182, 1889; *C. f. Bakt.*, i., 502, 1885.
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- 1014-15. Gayon and Dubourg., *Ann. Inst. Past.*, i., 525, 1886; *C. R.*, cx., 865, 1890.
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1025. Juhler, *ibid.* [2], i., 326.
1026. Kayser, *Ann. Inst. Pasteur*, v., 395, 1891.
1027. Klöcker and Schionning, *C. f. Bakt.* [2], i., 777.
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 1033. Lavoisier, *Ann. d. Chim.*, ii., 238, 1789; xxxvi., 116.
 1034. Lechartier and Bellamy, *C. R.*, lxi., 466; lxxv., 1204; lxxix., 949, 1006 (Z).
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 1036-37. Lindet, *C. R.*, cvii., 182, 1888; cxii., 102, 1891.
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 1039. Linossier and Roux, *C. R.*, cx., 868.
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 1041. Lüdersdorff, *Poggend. Ann.*, lxxvii., 408.
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 1043. Mann, *Ann. Inst. Past.*, viii., 785, 1894.
 1044. Maumené, *C. R.*, lvii., 398, 1863.
 1045-46. Mayer, *Landw. Versuchsstat.*, xvi., 277; xxv., 301.
 1047-48. Mazé, *C. R.*, cxxviii., 1608; cxxx., 424, 1899-1900 (Z).
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 1072. Rey-Pailhade, *C. R.*, cxviii., 201.
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 1074. Roeser, *Ann. Inst. Past.*, vii., 41, 1893.
 1075. Sanfelice, *Z. f. Hyg.*, xix., 32, 394, 1896 (Pathol. yeast).
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XIX. Oxydases.

G = Glycolytic. U = Urea-producing.

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- 1105-6. Achard and Weil, *C. R. Soc. Biol.*, l., 139, 986, 1898 (G).
- 1107-8. Arthus, *Arch. d. Phys.* [5], iii., 425; iv., 337, 1891-2 (G).
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TABLE OF THE MOST FREQUENT ABBREVIATIONS.

<i>Amer. Chem. J.</i>	= American Chemical Journal.
<i>Ann. d. Chim.</i>	= Annales de Chimie.
<i>Ann. Chim. Phys.</i>	= Annales de Chimie et Physique.
<i>Ann. Chem. Pharm.</i>	} = Liebig's Annalen der Chemie und Pharmacie.
<i>Lieb. Ann.</i>	
<i>Ann. Pharm. Chim.</i>	= Annales de Pharmacie et Chimie.
<i>Ann. Inst. Pasteur</i>	= Annales de l'Institute Pasteur.
<i>Arch. d. Phys.</i>	= Archives de Physiologie.
<i>Arch. f. exp. Pathol.</i>	= Archiv für experimentelle Pathologie und Pharmakologie.
<i>Arch. f. Hyg.</i>	= Archiv für Hygiene.
<i>Arch. f. klin. Med.</i>	= Archiv für klinische Medicin.
<i>Ber. d. d. chem. Gesells.</i>	= Berichte der deutschen chemischen Gesellschaft.
<i>Biol. Ctbl.</i>	= Biologisches Centralblatt.
<i>Bull. Soc. Chim.</i>	= Bulletin de la Societ� Chimique, Paris.
<i>C. f. Bakt.</i>	= Centralblatt für Bakteriologie.
<i>C. f. Phys.</i>	= Centralblatt f. Physiologie.
<i>C. med. Wiss.</i>	= Centralblatt für die medicinischen Wissenschaften.
<i>Chem. Centrbl.</i>	= Chemisches Centralblatt.
<i>Comptes Rendus</i>	= Comptes Rendus de l'Academie des Sciences.
<i>C. R. Soc. Biol.</i>	= Comptes Rendus de la Societ� de Biologie.
<i>Du Bois Arch.</i>	= Archiv für Anatomie und Physiologie, Physiol. Section.
<i>Jahrb. wiss. Bot.</i>	= Pringsheim's Jahrbücher für wissenschaftliche Botanik.
<i>J. Chem. Soc.</i>	= Journal of the Chemical Society, London.
<i>J. of Phys.</i>	= Journal of Physiology.
<i>J. pr. Ch.</i>	= Journal für practische Chemie.
<i>Koch's Jb.</i>	= A. Koch's Jahresbericht ü. d. Fortschr. auf d. Gebiete d. G�hrungsorganismen.
<i>Landw. Jb.</i>	= Landwirthschaftliche Jahrbücher von Thiel.
<i>Landw. Vers.</i>	= Landwirthschaftliche Versuchsstationen.
<i>Maly's Jb.</i>	= Maly's Jahresbericht für Thierchemie.
<i>Nat. Rdsch.</i>	= Naturwissenschaftliche Rundschau.
<i>N.S.</i>	= New Series.
<i>Pflüg. Arch.</i>	= Pflüger's Archiv für die gesammte Physiologie.
<i>Phil. Trans.</i>	= Philosophical Transactions of the Royal Society, London.
<i>Proc. Roy. Soc.</i>	= Proceedings of the Royal Society, London.
<i>Scand. A. f. Phys.</i>	= Scandinavisches Archiv f. Physiologie.
<i>Schmidt's Jb.</i>	= Schmidt's Jahrbuch f. d. gesammte Medicin.
<i>Virch. Arch.</i>	= Virchow's Archiv für Pathologie.
<i>Z. f. Biol.</i>	= Zeitschrift für Biologie.
<i>Z. klin. Med.</i>	= Zeitschrift für klinische Medicin.
<i>Z. physiol. Ch.</i>	= Zeitschrift für physiologische Chemie.
<i>Z. ges. Brauw.</i>	= Zeitschrift für das gesammte Brauwesen.

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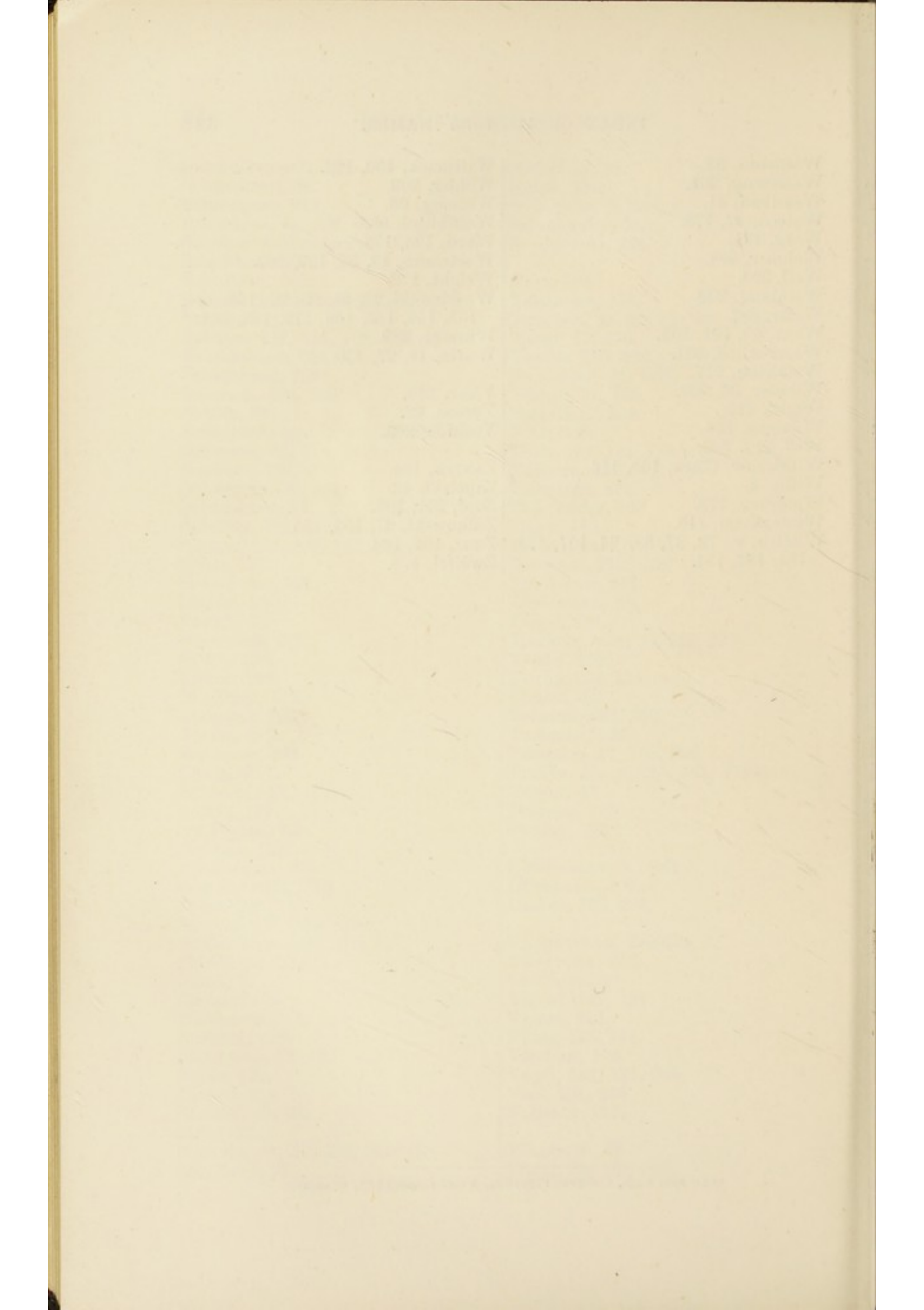
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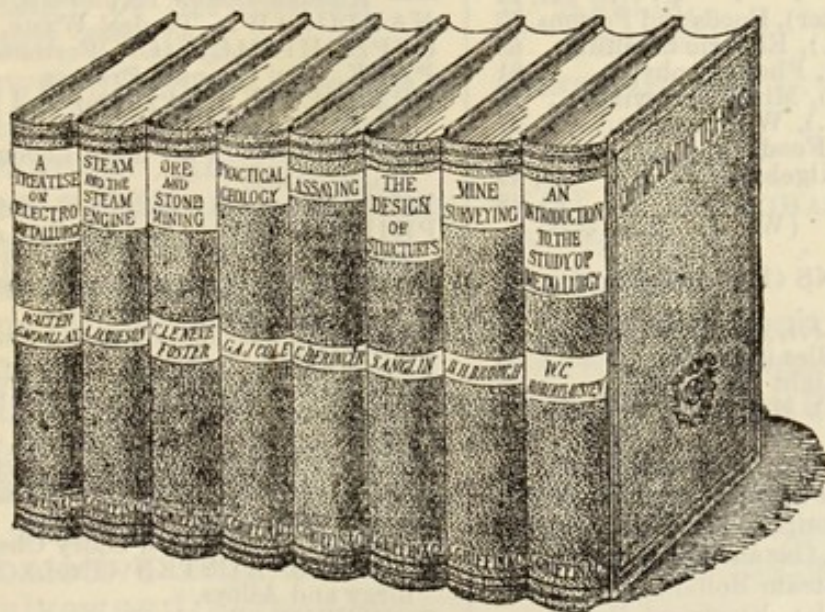
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
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COMPILED FROM OFFICIAL SOURCES.

*Comprising (together with other Official Information) **LISTS of the PAPERS read during the Session 1900-1901 before all the LEADING SOCIETIES throughout the Kingdom engaged in the following Departments of Research:—***

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| § 1. Science Generally: <i>i.e.</i> , Societies occupying themselves with several Branches of Science, or with Science and Literature jointly. | § 6. Economic Science and Statistics. |
| § 2. Mathematics and Physics. | § 7. Mechanical Science, Engineering, and Architecture. |
| § 3. Chemistry and Photography. | § 8. Naval and Military Science. |
| § 4. Geology, Geography, and Mineralogy. | § 9. Agriculture and Horticulture. |
| § 5. Biology, including Microscopy and Anthropology. | § 10. Law. |
| | § 11. Literature. |
| | § 12. Psychology. |
| | § 13. Archæology. |
| | § 14. MEDICINE. |

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* * Owing to the alteration of the date of publication of this work, so as to make its appearance coincident with the commencement of the Sessional year, the present volume contains (as far as possible) lists of all papers read between January 1, 1900, and June 30, 1901 (eighteen months).

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