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FIFTY YEARS OF PLANT PHYSIOLOGY

N.V. VAN DE GARDE & CO'S DRUKKERIJ, ZALTBOMMEL

FIFTY YEARS
OF
PLANT PHYSIOLOGY

BY

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WITH AN INTRODUCTION
BY

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1949
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Aan de Nagedachtenis van mijn vrouw,
de trouwe hulp bij mijn wetenschappelijk werk.

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INTRODUCTION

It is with a great deal of pleasure that I have read the manuscript of this book by the present dean of plant physiologists in the Netherlands. The title might have been: Textbook of Plant Physiology on a Historical Basis. Yet it is a natural continuation of the History of Botany by Sachs covering the field up to 1860, and the book by Reynolds Green taking up the period of 1860—1900. Professor Weevers's book reviews the development of Plant Physiology from 1895—1945.

It is fortunate that Professor Weevers decided to have his manuscript translated into English, because now it will not only reach a much wider circle of readers, but it will serve as an introduction to much continental European botanical literature, which is too often overlooked in the English-speaking countries. Such oversight is understandable if one realizes that in the last 50 years approximately 40,000 publications in the field of Plant Physiology appeared with well over half a million printed pages! This also makes it obvious why Professor Weevers had to make a selection of the literature and discuss those contributions which seemed to be most significant. In this way he has succeeded in giving a well-rounded presentation of the field of Plant Physiology, which is complementary to textbooks of Plant Physiology written in English.

Much stress is laid — deliberately — on contributions by Netherlands botanists; about one-fifth of all the research mentioned in this book was carried out the in Netherlands. Although this is more than actual contribution to the general knowledge of plant physiology, the Dutch can be proud of the quality and quantity of their research in this field, and botanists of other countries will welcome a review of this work. Some of it, such as the excellent work of van Herk, or the monumental series of investigations by Blaauw and collaborators, though fundamental, are almost unknown outside the borders of the Netherlands.

The translation of this book has been very literal, so that many Dutch figures of speech are retained, and thus the book has a distinctly Dutch flavor. I may be biased, but I think this lends color to the book. It also makes it possible to follow more closely the author's reasoning.

January, 1948 F. W. WENT

PREFACE

There are just a few words with which I should like to launch this book on its course: words of thanks, first of all to the translator Mrs. A. J. M. J. Rant of Hounslow, Middlesex, for the painstaking way in which she performed her difficult task; to my colleague. Professor F. W. Went of the "California Institute of Technology", for his true kindness in reading the English text, more especially in order to check the correctness of the scientific terms — though he also gave me many helpful hints concerning the latest American publications — and last but not least in writing his Introduction.

Also words of thanks to my former collaborator, now my successor, Professor A. W. H. van Herk, of the University of Amsterdam, who very kindly cooperated in the writing of a few sections of Chapter II and lastly to my son Professor Th. Weevers Litt, D. of the University of London who translated a number of quotations and passages subsequently inserted.

I also wish to thank all those who by their contributions to the present given to me on my retirement helped to render possible this translation of my book.

No one knows better than I how many things are passed over in this book and indeed inevitably so. For it would have been impossible to mention all the thousands of publications on plant physiology of the past fifty years, both in view of the book's scope and of its readibility. A selection had to be made, and this by its very nature must involve personal, if not arbitrary decisions. Nearly every reader will probably find some point at which he would have selected differently. I have endeavoured wherever possible to mention those authors and publications that in some measure contributed to the growth of plant physiology up to the year 1945.

Owing to the original plan of having this book published in Dutch the work done by plant physiologists in the Netherlands has been accorded rather more space.

TH. WEEVERS

Chapter I

INTRODUCTION

(A) Outline of the Book.

One of the few privileges which age confers upon us is the power of seeing the past in perspective. It enables us to survey all that we have experienced as if from a lofty point of vantage, and to discern a line of development in a body of research which may have seemed to lack connection at the time of its appearance. One naturally wishes others to share the pleasure of a novel experience such as this. That is why I have felt tempted to write the present survey of the development of plant physiology since 1895. I have chosen that particular year as my starting point for various reasons. To begin with, there is the personal one, that it was then that I entered upon my career as a University student of botany, and began to attend the lectures on plant physiology by Hugo de Vries [687].

Now in those days in Holland the freshman about to begin the study of biology was nothing like as learned a person as at present. Of plant physiology and anatomy I knew next to nothing; the only field in which I felt moderately at home was the flora of our country, and it was this knowledge that had awakened my interest in botany. It may be readily imagined how entirely new and fascinating was the world that opened out to me in those first lectures by Hugo de Vries. I can still picture him as he stood there before us in the stuffy little lecture theatre in the Botanical Laboratory of the University of Amsterdam, initiating us in such a masterly fashion in the mysteries of the anatomy and physiology of plants.

In that same year 1895, moreover, the last edition appeared of de Vries's Textbook of Botany (Part I, Anatomy and Physiology), revised by himself, which book has had such a far-reaching effect on the development of plant physiology in the Netherlands.

At approximately the same time (1897), W. Pfeffer [504] published the first part of his "Handbuch der Pflanzenphysiologie" which was to dominate this branch of botany for many years to come.

I think the most practical way of setting out the history of plant

physiology will be to divide the subject into its various branches on the lines adopted by DE VRIES in his Textbook and to draw a picture of its development during the past fifty years by reviewing the history of each of these branches separately. A purely chronological order would, I feel sure, have a confusing effect, which is no doubt the reason why J. REYNOLDS GREEN [541] in his "History of Botany, as well as M. MÖBIUS [433] in his "Geschichte der Botanik" adopted a similar method. In order to proceed in this way, however, it will be necessary first to give a rough outline of the development of plant physiology as a whole up to 1895, the proposed starting point of my detailed account. This will, moreover help to bring out the fact that about that time a certain phase in the development of plant physiology closed and a new phase begins. For it was in 1897, that JULIUS SACHS [565] died and his death ended a chapter of plant physiology which was, as it were, centred round his person.

(B) Short Summary of Plant Physiology till 1895.

Plant physiology is an experimental science and is, therefore, based on experiments; as long as physiology was founded on the speculative views of ARISTOTLE [11], its progress was nil. One example may suffice to prove this contention: ARISTOTLE tried to understand the nature of the plant by comparing it to that of the animal; the soil was supposed to function as the stomach and the bowels ("ta koidia") of the plant which was thought to absorb, by means of its roots, the food that had been prepared for it. (sic)

During the Middle Ages the advancement of plant physiology was negligeable. It was not until the days of the Renaissance that the interest in living nature revived. The Dutch scientist, J. B. VAN HELMONT [249], a physician and alchemist who worked in Brussels, was the first to carry out deliberate physiological experiments on plants. He put 300 pounds of dry soil into a vat in which he planted a willow branch weighing 5 pounds. The vat was watered with what VAN HELMONT called pure water and protected against dust. Five years later the branch had grown into a tree weighing 164 pounds, while the weight of the dry soil had decreased by only 2/5 th pound The conclusion drawn by VAN HELMONT was the obvious one; the increase in weight of the branch was due to the water or, in other words, the plant formed its component parts out of water.

The ignorance of physics and chemistry which prevailed at the time, renders this conclusion understandable; nevertheless, it is shown by the work of J. WOODWARD [744], a London professor who lived about a century later, that even without marked progress in these branches of science more accurate conclusions might be arrived at by exercising greater care in the carrying out of experiments. WOODWARD proved experimentally that plants would grow better in river water than in

rain water, especially when the former is turbid and, therefore, contains minute solid particles.

The progress made in other branches of natural science sometimes had a stimulating, at other times a retarding effect on the development of plant physiology. It was beneficial in the case of the observations made by MARIOTTE [408] who applied the new conceptions of physics in his explanation of the movement of water inside the plant; the opposite effect was seen in the work done by ST. HALES [225] when WILLIAM HARVEY'S [244] discovery of blood circulation led him to assume the existence in the higher plants of a kind of circulation of the sap comparable to the circulation of blood.

Before proceeding to a brief discussion of HALES's work, I ought to mention that MALPIGHI ([404]), rather by dint of astute reasoning than by experiments had come to the conclusion that assimilation of the substances supplied by the vessels took place in the leaves. MALPIGHI had observed that a seedling would not grow any more after the cotyledons had been removed; hence he argued that these cotyledons must be the means of supplying the food to the growing plant and, realising on morphological grounds that the cotyledons were leaves, he concluded that the ordinary leaves must have the same function. MALPIGHI also had a fairly good idea of the course followed by the water in the plant, though he was not quite clear as to which part of the wood served for its translocation.

It was not until the beginning of the 18th century that HALES conducted his interesting experiments concerning transpiration and the movement of water in the plant. MALPIGHI still spoke of air tubes, hence the word tracheae, while the water was supposed to move along the fibres; HALES, however, had a good conception of the vessels by which the water is conveyed from the roots to the leaves, although the functional difference between wood and bast was not quite clear to him. He tried to explain the phenomena which he had observed by deduction from physical laws; the absorption of water by the roots, for instance, he compared with capillary attraction. Through experiments he was familiar with the suction-pressure of leaves, the so-called "vis a fronte", and also with the pressure from the roots, the "vis a tergo" of the translocation of water. These were termed thus, because the former drew up the water, as it were, from the front, while the latter was supposed to push the water upwards from behind.

HALES did not realise, however, that once assimilated the various substances, such as sugars, are conveyed through the bast; his lack of insight into the function of the leaves led him astray. He did understand that light had something to do with it, but his conceptions of the processes taking place in the leaf were vague. This could, indeed, hardly be otherwise as long as the composition of the atmosphere remained unknown and the process of photosynthesis was not understood.

A first step forward in this connection was made by PRIESTLY [520] in

1774 by the discovery of oxygen, when it was also found that green plants will improve air which has been vitiated by the presence of humans or animals. A year after, however, K. W. SCHEELE [574] arrived at a directly opposite result, viz. that even green plants can vitiate the air. It was the Dutch scientist JAN INGENHOUSZ [286] — originally physician at Breda, later court physician to the Empress Maria Theresia in Vienna — who threw some light on the subject. In 1779 he published his "Experiments upon Vegetables discovering their great power of purifying the common air in the sunshine and of injuring it in the shade and at night." In that year INGENHOUSZ still adhered to the phlogiston theory and spoke of dephlogisticated air and foul air, but presently the work of A. L. LAVOISIER [367] came to invalidate the phlogiston theory. It was found that carbonic acid was the compound of carbon and oxygen which is present in so-called foul air. In 1796 INGENHOUSZ published a more modern version of his work, in which he observed that oxygen is produced by green plants exposed to light. provided they are kept in an atmosphere containing carbonic acid (carbon dioxide). This publication shows, moreover, that INGENHOUSZ had a clear conception of the respiratory process in the dark, but it does not appear whether he realised that during exposition of the plant to light this respiratory process takes place in the green parts also.

The title of INGENHOUSZ's publication already indicates that, for him, the primary interest of the carbon dioxide assimilation lay in the cosmic process, i.e. the purification of the atmosphere, while its significance in regard to the formation of organic matter and the consolidation of energy was underrated by him. When in Napoleonic days homage was paid to the merits of INGENHOUSZ, he was — characteristically — praised for having established that "every green plant, even the smallest and most poisonous one, is useful to man, because it purifies the atmosphere". This is quite correct, but the fact that, in addition, all our food is formed by that same process of photosynthesis, was being overlooked. The first to realise this was the brilliant plant physiologist N. TH. DE SAUSSURE [569] of Geneva. In his "Recherches Chimiques sur la Végétation", published about 1800, he cited experiments to prove that:

(1) the plant obtained carbon from the carbonic acid in the atmosphere;
(2) the elements hydrogen and oxygen were assimilated with the carbon, in the proportion in which they were present in the water;
(3) the increase in the dry weight of the photosynthesizing plant was due to the processes referred to in (1) and (2) above;
(4) in addition to this a further condition necessary for growth was the absorption of nitrogen and a few other elements which the plant imbibed from the soil along with the water in the form of mineral salts; this nitrogen was supposed to be derived, in the last instance, from the products of the decomposition of vegetable and animal substances.

(5) the less viscous these compounds, the better they were absorbed;
(6) respiration was essential to the life of the plant; it exhaled carbonic acid and water. Growing plants had a stronger respiration than portions of plants that were resting. By means of respiration energy was obtained.

Clearly, this was a sound basis for further investigation into the subject of metabolism. And when it is, furthermore, remembered that the study of the translocation of the various substances, which had been started so ably by HALES [225] was continued by T. A. KNIGHT [335] who argued that the products formed in the leaves were conveyed along the bast, it may be said that at the beginning of the 19th century the physiology of metabolism was developing rapidly. In view of this, it is all the more remarkable that thereupon a period of stagnation and even retrogression set in, during which DE SAUSSURE's views were ignored and even considered unsound. The humus theory, for instance, came to the fore again, according to which all the substances required for the nutrition of the plant were supposed to be derived from the soil; this was, therefore, also believed to be the case with carbon, a theory already refuted by INGENHOUSZ.

A few quotations from a leading textbook, published 1832 by A. P. DE CANDOLLE [105] will demonstrate this temporary setback of plant physiology. He says e.g.: "The spongioles of the roots (root caps) are actively contractile and, by means of the capillarity and the hygroscopic qualities of their tissue, they suck up the surrounding water together with salts, organic and gaseous elements. By means of the contractility of the cells and vessels the moisture is conveyed to the leaves via the intracellulars (sic). The crude (unconverted) sap which reaches the leaves, there comes into contact with the sunlight which during the day decomposes the carbondioxide gas derived from the moisture in the root or from the air. The carbon element remains in the plant and forms a kind of gum, composed of one atom of water and one atom of carbon which may be converted into starch or sugar. The alimentary sap thus prepared goes from the leaves to the roots overnight; in the case of some plants it travels along the bast and the sap-wood, in the case of others it is conveyed along the wood".

In the year 1838 F. J. F. MEYEN [424] stated that in plants there is no respiration such as is found in animals, and at about the same time C. L. TREVIRANUS [658] doubted the decomposition of carbonic acid and propounded the theory that all the carbon in the plant was derived from the humus in the soil. By assimilation, TREVIRANUS understood the conversion of the unpurified sap referred to by MALPIGHI. In 1837 referring to the work done by TREVIRANUS, M. J. SCHLEIDEN [576] said: "dass die Zeit von MALPIGHI bis TREVIRANUS eine sterile Wüste sei", whilst of TREVIRANUS himself he stated: „dass er wie ein Meteor durch die Nacht strahlte."

It would lead us too far if we were to attempt a thorough analysis

of this phenomenon of the retrogression of plant physiology. MÖBIUS in his "Geschichte der Botanik" blamed the contemporary philosophy of nature which attributed almost everything to a mystical vital force. In my opinion, it was also the outcome of the disproportionate growth of plant physiology during the previous decades. Then, important results were attained by methods which were borrowed chiefly from physics and chemistry, but while by these methods the various processes viewed as a whole, were explained, the structure of the substratum where these processes took place remained obscure. This could not fail to produce false conclusions, and it may be said it was not until the cellular structure of that substratum had been fathomed that plant physiology again showed an upward curve True, there was some progress made in connection with particular aspects of it, e.g. by E. H. J. DUTROCHET's [149] investigations on the subject of osmosis, but they did not affect the science as a whole.

The change, when it did come, came from the side of cytology; the nucleus in the epidermal cells of Orchids had already been observed by ROBERT BROWN [81] as early as 1833, but it was HUGO VON MOHL [435] who gave us an insight into the structure of the cell in general. It was he, who in 1846, gave to ,,die trübe, mit Körnchen gemengte Flüssigkeit von weisser Farbe, die ausser Primordialschlauch und Zellkern die Zelle mehr oder weniger erfüllt" [1]) the name of protoplasm. It was not until then, that a good idea was obtained of the cell, a term which had been used already in 1667 by R. HOOKE [269] on account of the similarity between the tissue of cork (the object studied by him) and the cells of a honeycomb. Cytology thereupon progressed with rapid strides; in 1838 SCHWANN [594] and SCHLEIDEN [576] had clearly stated that the cell constituted the essential foundation of the plant and this pronouncement was elaborated further by VON NÄGELI [449] who demonstrated that every new cell originated from an already existing cell, in other words the dictum of RUDOLF VIRCHOW [681]: "omnis cellula e cellula". This is not the place to go deeply into the subject of cytology; to us, its only interest lies in the extent to which it has been of use in the revival of plant physiology.

It took a considerable time for these new concepts to find general acceptance, and the central figure through whom all this found expression was JULIUS SACHS [565]. But before proceeding to a short discussion of his work, we must first devote a few words to those who preceded him.

In 1840 there appeared a book by J. VON LIEBIG [376], entitled "Organische Chemie in ihrer Anwendung auf Agricultur und Physiology", which had a far-reaching effect on plant physiology. He discarded the humus theory, according to which — as we stated earlier — all elements, including carbon, were supposed to be derived from humus. It did not state, however, which elements were absolutely essential to

[1]) "the turbid, viscous, whitish liquid, containing minute particles, which more or less fills the cell as well as the nucleus and the vacuole do"

the plant, and it was not until a few years afterwards that A. F. J. WIEGMANN [723] and A. L. POLSTORFF [514] published the results of their experimental investigations in response to the offer of a prize by the Göttinger Academy: plants obtained their normal nutriment only if they were able to absorb the ashes out of the soil in a state of solution. This answer, therefore; differed greatly from that given in 1800 in response to the offer of a prize by the Berlin Academy on the subject of the origin of the ash content of plants. At that time the answer, which received an award in gold, read as follows: "die Pflanzen erzeugen die in ihnen enthaltenen Aschenbestandteile selbst durch ihren Lebensprozess" (by their vital processes the plants produce themselves the ashes which are found in these plants).

As tho the nitrogen LIEBIG still believed the content of this element in the plant to be derived from ammonia, and it was by the work of J. B. D. BOUSSINGAULT [67] that the importance of nitrates to the plant was clearly proved. He also carried out the first experiments indicating that fixation of atmospheric nitrogen takes place during the development of Papilionaceae.

Progress was also made in respect of the assimilation of carbon. As early as 1819 J. PELLETIER [501] and J. B. CAVENTOU [107] had introduced the conception of chlorophyll, and investigations were instituted as to which light-rays played the leading part in photosynthesis. At first it was thought that these were the so-called chemical rays, the blue ones, but in 1836 C. G. B. DAUBENY [134] concluded that the yellow section of the spectrum was the most important. At about the same time, DUTROCHET [149] applied the nowadays so well-known method of counting the gas bubbles which rise from a sprig of *Elodea* when this is placed under water in a light of sufficient intensity. Again it was VON MOHL [435] who demonstrated that the chlorophyll in the cell was not scattered diffusely, but was present in granules — nowadays termed chloroplasts — in which the production of starch during photosynthesis was rendered visible by him with the aid of a solution of iodine.

Yet the leading textbook of those days in Germany, "Die Grundzüge der Botanik" by M. J. SCHLEIDEN [576] did not pay due attention to these observations. It was left to SACHS to summarize them all and to make them common property of the botanical world.

It is beyond the scope of this volume to go deeply into SACHS's work: this would entail a survey of the entire field of the plant physiology of those days. In his Würzburg laboratory he and his pupils worked on nearly every branch of it and thus the Würzburg university, which in 1868 had appointed him as full Professor, became the centre of the plant physiology of his time. Of his pupils I need only mention HUGO DE VRIES PFEFFER, GODLEWSKI [201] and VINES [680], to show the extent of his influence in Europe. SACHS's work reached its culmination about 1882, when he published his book, „Vorlesungen über Pflanzenphysiologie", and this date is so near the year 1895, which I have selected as my

startingpoint, that there would be no point in discussing in extenso the general aspect of plant physiology in 1882. Yet a few parts of SACHS's work must be discussed in greater detail.

(1) Assimilation of carbondioxide (photosynthesis). The results obtained by DE SAUSSURE [569] which had almost been forgotten, were brought to the fore again by SACHS. This he did by linking them up with the new findings of cytology. The fact that it is in the chloroplasts that photosynthesis takes place, was brought out clearly; so was the part played by the different sections of the spectrum in both the formation of chlorophyll and the supply of energy required for photosynthesis itself. SACHS also established the fact that starch is the first visible and best demonstrable product.

By means of DUTROCHET's gas bubble method he studied the intensity of photosynthesis in the various sections of the spectrum, and once again the conclusion arrived at was that the section from yellow to red was the most active, a subject which was to be followed up further by SACHS's pupil, W. PFEFFER [504]. SACHS did not go further into the question of the real essence of this carbon assimilation process or what the respective functions are of light and chlorophyll in this connection.

(2) The translocation of water. The question of the route along which water was conveyed had been regarded as settled since HALES's experiments; nothing was, however, known as yet of the forces causing this translocation; only unproved suppositions were made. Critical study on this point led SACHS to take the subject up again because DUTROCHET's observations on osmosis, which were to be continued and extended by HUGO DE VRIES and by PFEFFER, had thrown a different light on it.

It is curious that, after carefully weighing up the various possibilities, SACHS finally arrived at a conclusion which, at first sight, strikes one as very strange, viz. that the translocation of water must take place *in the wall* of the wood vessels. This imbibition theory, although argued by SACHS, failed to find favour among botanists, despite the prestige of the name of the author. Indeed, the theory seemed to be completely refuted by different experiments which showed that the translocation of water was obstructed when the interior of the vessels was filled up with gelatine or cocoa butter. Yet we shall find later that, now after more than 50 years, certain views are cropping up again in plant physiology, which agree to a remarkable extent with those of SACHS, although they concern only a special section of this water translocation.

(3) SACHS's views have proved equally vital in an entirely different branch of physiology. In his investigations and observations in respect of the origin of the flower, SACHS concluded that there were substances which he termed flower-forming substances and which, when brought to the growing point, would induce the origin of the flower structure. This view was, likewise, completely rejected by his contemporaries

and it was not until 50 years had passed and the phytohormones were discovered that this idea of JULIUS SACHS was brought to the fore again by F. W. WENT [710]. Nowadays the existence of flower-forming substances in the case of so-called short-day plants and long-day plants, if perhaps not strictly proved, is at least fairly generally accepted. If a short-day plant be grafted upon a long-day variety of the same species, not only the scion, but also the stock in the region of the graft will produce flower-buds when exposed to short-day illumination.

So here also we find in SACHS a kind of intuition, as it were, which led him to hold views that were not to meet with general acceptance until several decades later — in my opinion a sure proof of his genius.

We have now so nearly approached the time of the appearance of the 3rd edition of the Textbook by HUGO DE VRIES and C. A. J. A. OUDEMANS [484] (the latter wrote the sections on morphology and systematic botany), that there is no point in separately following up the development of plant physiology between 1882 and 1895; I can, therefore, now proceed to a detailed discussion of the subject-matter, in which I shall each time begin with a short summary of the respective chapter as outlined by DE VRIES.

CHAPTER II

RESPIRATION

(A) Introduction

At the very outset we are struck by the fact that, contrary to the order followed in most textbooks, DE VRIES [687] opens the discussion with the chapter on respiration. This method has its advantages and disadvantages: respiration being the most essential of all vital processes, it can be dealt with either at the beginning, as the most important chapter, or at the end by way of summary. HUGO DE VRIES clearly realised that respiration is inextricably bound up with life, as is evident from his summary:

(1) External respiration is the result of internal respiration;
(2) Internal respiration is a function of the living protoplasm and ceases at death;
(3) In the process of internal respiration the protoplasm constantly sets free certain substances and absorbs others;
(4) By the substances that are absorbed chemical tensions are continually set up in the protoplasm and are there transformed into vital forces, thus supplying the energy for the various activities of the living organism;
(5) The substances which the protoplasm absorbs to this end (except in the case of some of the lowest organisms) are oxygen and sugar; the substances given off are carbon dioxide and water.

When in accordance with our terminology the word "tensions" is translated into "energy", we can fully endorse all this, only the term "vital forces" remains obscure to us, probably as obscure as it was to DE VRIES himself.

In the exposition of the above — mentioned points in the textbook we are furthermore struck by the tendency towards an experimental, quantitative method which forcibly reminds one of the work done by DE SAUSSURE in the year 1804. The great advance, though, lies in the fact that everything is referred to the internal respiration which has its basis in the living protoplasm.

According to DE VRIES, in the great majority of cases protoplasmic

streaming ceases when no oxygen is available; the only exception being *Chara* in which this phenomenon of streaming was discovered by B. CORTI [121] in the year 1774. The motions of leaf-joints and petals also cease and growth is at a standstill; by lowering the tension of oxygen in the environment, most processes are proportionally retarded.

Closer research, however, will show that, though absorption of oxygen may be temporarily suspended the setting free of carbon dioxide will continue without a break as long as the tissue is alive. However we have to consider the fact that as a result of photosynthesis in the green plant which is exposed to light the liberation of carbon dioxide may be masked.

By comparing the respiratory intensity of germinating barley with that of clover, the former of which contains approximately twice the amount of nitrogen and during germination produces almost double the quantity of carbon dioxide of the latter, DE VRIES argues that the quantity of protoplasm determines the respiratory intensity. The quantity of protoplasm is, in DE VRIES's opinion, indicated by the nitrogen content. Although we would not consider this as binding evidence anymore, it is yet a brilliant thought which later was elaborated further by W. PALLADIN [494].

The fact that, generally, the quantity of oxygen that is absorbed during respiration equals the quantity of carbon dioxide which is given off and that, as appears from the decrease in weight, also water is produced in respiration, is dealt with quantitatively in the manner of DE SAUSSURE and illustrated by germinating experiments. On the subject of the germination of oleiferous seeds the textbook also agrees with that author. The fact that in this particular instance the quantity of oxygen absorbed during germination greatly exceeds the quantity of carbon dioxide that is given off, was at that time correctly interpreted as meaning that oil and fats are converted into sugars before dissimilation.

That respiration is the source of the heat produced in some parts of the plant and that this heat production is approximately proportionate to the quantity of carbon dioxide that is produced, was recorded by DE SAUSSURE in the year 1822. With the aid of a thermo-couple, DUTROCHET [149] could demonstrate experimentally that there is also a rise in temperature in the buds and leaves. In the spadix of the *Araceae* this heat production had been studied repeatedly, first by DE LAMARCK [362] in the year 1777, later in Holland by H. W. DE VRIESE [689] and G. VROLIK [690]. In the textbook by HUGO DE VRIES several cases of such rises in temperature are mentioned; in regard to some of them — for instance in seeds or leaves — the present-day theory is that, if they reach a degree of, say 10° to 20° C, they are the result of bacterial processes. It is only in flowers, particularly in the *Araceae* mentioned above, that a rise in temperature of this kind is the result of respiration in that particular part of the plant.

A characteristic feature of the school of thought that prevailed about

the time at which we begin our more detailed survey of plant physiology is the sharp distinction between plasmatic and aplasmatic metabolism. In the former category are grouped all those processes in which the molecules of the living protoplasm themselves undergo chemical changes, termed vital processes; the latter comprises those processes in which the molecules of the protoplasm are not regarded as taking a direct part. The action of hydrolytic enzymes in particular was viewed as an instance of aplasmatic metabolism. Hence, formation of invertase was a plasmatic process and the breaking down of sucrose by means of this invertase was an aplasmatic process. Fermentation of monosaccharides by yeast, *Saccharomyces* spec. was again regarded as a plasmatic process, as it was supposed to be bound up with the living protoplasm.

Summarizing, we may say of this chapter of the textbook that we are struck by the fact that, in spite of a wide factual knowledge and despite the stress laid on the indissoluble connection between respiration and the life of the protoplast, there is a total lack of insight into the nature of the respiratory process.

It must be admitted that in this respect the textbook by DE VRIES shows a lacuna since it fails to give a complete picture of the knowledge attained at the time — that is, with regard to intramolecular respiration.

(B) Chemistry of Respiration and Fermentation; Research before the Year 1921.

As early as 1819, it was observed by K. G. GRISCHOW [213] that in mushrooms the quantity of carbon dioxide which is given off, at times greatly exceeds the quantity of oxygen which is absorbed; SACHS found later that in an environment devoid of oxygen, such as the vacuum of TORRICELLI, various parts of a plant produce carbon dioxide. In 1875 an observation by the animal physiologist E. F. W. PFLÜGER [505] attracted general attention: PFLÜGER found that frogs can keep alive and exhale carbon dioxide for a while in an environment devoid of oxygen. This was taken as an indication that the molecules of the organic substances of the animal were broken down even without admission of oxygen, in such a way as to produce carbon-dioxide. In this connection PFLÜGER spoke of intramolecular respiration.

SACHS realised that this process would also take place in plants, and at his instigation J. WORTMANN [745] subjected the question to close research. With various control plants it was found that during the first few hours intramolecular respiration produces approximately as much carbon dioxide in the objects used as did the normal respiration, though this production soon decreases. SACHS drew the conclusion that intramolecular respiration was an abnormal phenomenon and he opposed PFEFFER's view that both normal and intramolecular respiration were processes similar to that of fermentation. Though it did

not escape the keen eye of SACHS that intramolecular respiration constituted evidence of the fact that, in its initial stages, the respiratory process can take place independent of oxygen, he did not have a clear insight into the connection between fermentation, the normal and the intramolecular respiration. The latter process was called anaerobic respiration by KOSTYTSCHEW [344] and this term will henceforth be used here.

Though PFEFFER did point out the similarity and close connection between respiration and fermentation, arguing that they had one and the same root, he was unable to specify this further or to supply an answer to the question whether ethyl alcohol, which arises in fermentation, is also formed in normal respiration but immediately broken down. In anaerobic respiration something of the kind was to be expected as it had been demonstrated by LOUIS PASTEUR [497] that in the inner parts of fleshy fruits alcohol is produced when the tension of oxygen is insufficient. PFEFFER [504] pointed out that certain species of the genus *Mucor* growing on some substrates are observed to have a perfectly normal respiration, while after submersion in solutions of sugar fermentation takes place and the cells tend to assume more or less the character of ordinary yeast, *Saccharomyces cerevisiae.*

Here we must make some reference to fermentation, particularly alcoholic fermentation. In the year 1837 C. CAGNIARD DE LA TOUR [103] observed for the first time that in this process organisms are concerned; TH. SCHWANN [594] proved this more strictly by demonstrating that fermentation ceased if these organisms were killed. There remained, however, the important question of how to interpret the action of these organisms. By J. J. BERZELIUS [46] and E. MITSCHERLICH [431], both chemists, the process was regarded as a sort of surface action, analogous to that of platinum sponge. LIEBIG [376] regarded it as a ferment action and ignored the living organisms: according to him it was a matter of the decomposition of proteins involving the sugars in the process. In the year 1858 M. TRAUBE [656] still maintained that this ferment was formed by decomposition of proteins.

It was PASTEUR who gave us a clear insight into the matter. The idea of SCHWANN that alcoholic fermentation was a function of living organisms was worked out further by him and he succeeded in getting this view accepted by the scientific world. Without the admission of air, which contains germs, a solution of sugar at normal temperature will remain unaltered. In the presence of oxygen, said PASTEUR, the cells of *Saccharomyces* breathe in the same way as other organisms, but when no oxygen is available the yeast derives this element from the carbohydrate in solution: "la fermentation c'est la vie sans air". LIEBIG lost the battle, but he maintained to the end that the vital functions of yeast and the chemical processes by which alcohol is produced were independent of each other. Fermentation, says LIEBIG occurs only when the yeast has ceased to grow.

This is how matters stood in 1895, and it was not until the year 1897 that E. BUCHNER [87] took the next step towards clearer insight into the phenomena of fermentation and respiration. He discovered that, after grinding yeast with silver sand or "kieselguhr", it was possible by strong pressure (approx. 200 atmospheres) to obtain a clear juice which no longer contained any yeast cells, but which still retained the property of fermenting a solution of glucose. BUCHNER termed the substance which brought about this particular property of the juice, "zymase", and explained that this zymase was unable to diffuse outwards out of the intact and living yeast cells, but that this became possible only when the cells were pulverised. A similar juice was obtained also in other ways, e.g. with the aid of acetone, and although objections were raised, e.g. that the juice had not been free from living cells, H. VON EULER [163] finally proved BUCHNER's view to be correct. The importance of his discovery lies in the fact, that now for the first time a method was found of breaking down sugar into carbon dioxide and alcohol, which was not bound to the living protoplasm or, in the terminology used earlier herein, fermentation was found to be a process of aplasmatic metabolism.

Attention should be given to the facts observed some years later by BUCHNER, HAHN [224] and others. Glucose is more strongly fermented by this press-juice if natriumphosphate is added. HARDEN [234] and YOUNG [748] stated in 1905 the production of an organic phosphoric compound which proved to be fructofuranose-1-6-biphosphoric ester, the socalled HARDEN-YOUNG ester.

As long as glucose and anorganic phosphate, added both in aequimolar quantities are present in excess a close relation exists between the quantities of fermented and of esterified glucose. This relation can be expressed in the following equation.

$$2C_6H_{12}O_6 + 2H_3PO_4 = 2CO_2 + 2C_2H_5OH + 2H_2O + \\ + C_6H_{10}O_4(OPO_3H_2)_2 \quad (I)$$

However if glucose and phosphate are nearly consumed the hexosebiphosphate is again split up.

$$C_6H_{10}O_4(OPO_3H_2)_2 + 2H_2O = C_6H_{12}O_6 + 2H_3PO_4 \quad (II)$$

In the opinion of HARDEN and YOUNG both processes, phosphorylation of glucose (I) and dephosphorylation of glucose (II) take place continually in the living yeastcell. Alcoholic fermentation could be the result of these two processes combined.

This hypothesis proved to be too simple; the idea of an intracellular circulation of phosphoric acid in fermentation was of fundamental importance however. Some years later the biochemist C. NEUBERG [456] gave us entirely new methods for studying the action of zymase. If other substances existed which could be rapidly fermented by zymase, there was every possibility that such substances might play a part as intermediate compounds in the fermentation of the sugar. It was then

found that a solution of pyruvic acid can be split up by zymase, and NEUBERG attributed this fact to the presence of the enzyme carboxylase in the zymase complex. This carboxylase was thought to split off carbon dioxide from the carboxyl group of pyruvic acid.

$$CH_3COCOOH \rightarrow CH_3CHO + CO_2$$

NEUBERG also considered the problem from another angle and looked for substances which during the process of fermentation appear in small quantities in the normal course of events, but which in special circumstances may become the final product of the fermentation. It had been found that by the use of substances such as $NaHSO_3$ large quantities of acetaldehyde (CH_3CHO) can be obtained in fermentation. This acetaldehyde was also to be regarded as an intermediate stage of fermentation, while by removal of this acetaldehyde large quantities of glycerol arise, a substance of which normally only a small percentage is formed. By making use of these data NEUBERG arrived at the following picture of the process of fermentation:

$$C_6H_{12}O_6 \rightarrow 2H_2O + 2CH_3COCHO \text{ (pyruvic aldehyde)} \quad (I)$$

$$2CH_3COCHO + 2H_2O \rightarrow CH_2COCOOH + CH_2OHCHOHCH_2OH \text{ (glycerol)} \quad (II)$$

$$CH_3COCOOH \rightarrow CO_2 + CH_3CHO \text{ (acetaldehyde)} \quad (III)$$

$$CH_3CHO + CH_3COCHO + H_2O \rightarrow CH_3COCOOH + CH_3CH_2OH \text{ (ethyl alcohol)} \quad (IV)$$

According to NEUBERG, therefore, once acetaldehyde has been formed, it will, with the pyruvic aldehyde in accordance with (IV), repeatedly yield — by a so called CANNIZZARO[106] reaction — alcohol and pyruvic acid, thus enabling the reaction to continue. According to this conception glycerol will arise only at the beginning and in small quantity. If the acetaldehyde is removed, equation (II) must follow and the quantity of glycerol will increase. For this theory to be acceptable, it should also prove possible to isolate the pyruvic aldehyde as an intermediate compound. This indeed could be done, for it was found that in fermentation a part was played by enzymes which are easily dissociable compounds. They dissociate in the so-called co-enzyme which can resist a temperature. of circa 100° C and in the colloidal protein which is denaturated at this temperature. Often the co-enzyme (agon) can be separated from the protein (pheron) by dialysis. If this separation has taken place no more fermentation of glucose occurs; phosphorylated sugars can still be fermented but the breakdown will come to a standstill at a stage where the pyruvic aldehyde is present.

I thought it useful to go into this matter somewhat more deeply, not because NEUBERG's theory has given us the final explanation of the process of alcoholic fermentation — we shall see later that these views have since become modified in several respects — but because it is a

good indication of the way in which solution of those problems may be attempted and also of the important part played in this respect by different biochemists.

Now we shall leave the subject of alcoholic fermentation for a while in order to trace the influence of these discoveries on the study of normal respiration.

When BUCHNER had found that zymase can convert a solution of sugar into carbon dioxide and alcohol, it was natural that attempts should be made to find out if something of this kind also occurred in the plants with a normal respiration.

There is as we have seen earlier, very intensive respiration in the spadix of the *Araceae*, hence it was natural for these plants to be selected as the object of these investigations. In the year 1900 HAHN [224] prepared from this object, *Arum* spec. a press juice which was capable of breaking down a solution of glucose; a curious point was that in this case carbon dioxide, but no ethyl alcohol was produced. With the spadix of *Sauromatum* spec. I obtained a similar result, both in atmospheric air and in an atmosphere of hydrogen. I found moreover, that besides carbon dioxide, organic acids were formed, a fact which pointed to the action of an enzyme different from zymase. At that time objects were already known in which in anaerobic respiration carbon dioxide was produced but not alcohol, e.g. the common mushroom, *Psalliota campestris*, and the potato.

The Russian physiologist PALLADIN [494] examined various objects by means of the freezing method. When, for instance, etiolated seedlings of the broad bean, *Vicia Faba*, are cooled to a temperature of —22°C by means of a freezing mixture, the seedlings will die, but the enzyme complex is not destroyed. This is proved in the following manner: if these seedlings are placed in a solution of glucose, the latter will undergo decomposition; in an environment which contains oxygen carbon dioxide and water will be formed, but in an anaerobic environment intramolecular or anaerobic respiration will occur. That the tissue was really dead was proved not only by the blackening which set in but also by the fact that the stimulating effect which a solution of quinine has on living cells was lacking. The blackening showed that, though the enzymes were undamaged, the protoplasm which coordinates enzyme action had died. It is true that this anaerobic respiration can be intensified by the addition of phosphates, but this is due to their chemical action in the decomposition of the sugars.

In the same way as described above for yeast, it can be demonstrated that acetaldehyde is also formed in anaerobic respiration — an additional proof of the similarity between the two processes.

We now have to consider the problem of respiration from another angle. From the outset the great difficulty in obtaining a better insight into the respiratory process lay in the fact that the substances which serve as a source of energy in the process — in this case glucose — lack

the property of being oxidizable by the oxygen in the air at an indoor temperature.

Monosaccharides lack that property, as do those substances which in the light of the above, may be regarded as intermediate compounds in the respiratory process, such as pyruvic acid, acetaldehyde or ethyl alcohol. Hence the inclination to assume the co-operation of autoxidable substances by means of which the oxygen in the air — in itself inactive — would be transformed into an active agent. As early as 1863 this idea was propounded by C. F. SCHÖNBEIN [582] who had in mind the co-operation of ozone; in the year 1882 M. TRAUBE [656] pointed to the possible co-operation of peroxides, particularly hydrogen peroxide. In the year 1897 this idea TRAUBE's was worked out further by A. BACH [24] and R. CHODAT [112] as follows. They supposed that the plant possessed a system with a specifically oxidizing action, capable of oxidizing certain substances. Through the intake of oxygen from the air peroxides (AO_2) would arise which in the presence of a peroxidase would form the oxidizing system; in other words, with peroxidase the peroxide would produce oxygen in statu nascendi.

It should then prove possible to demonstrate the presence of such an oxidase system by adding to the pulverized tissue a colourless chromogen which upon oxidation would yield a coloured product, e.g. emulsion of guaiacum which assumes a blue colour. If this conception was correct, it should be possible to break down or to split up this oxidase system into its two components, and it was believed by some, e.g. by M. WHELDALE [718], that they had succeeded in doing so.

Systems of this kind were found in a number of plants usually in those with some aromatic product or other, and in these plants a typical colouring did, in fact, arise in necrobiosis, in which the protoplasm is dead but the enzymes are undamaged. A serious objection was that the species in which such systems are lacking greatly outnumber the others. PALLADIN ([444]) tried to overcome this difficulty by another hypothesis. He assumed that in all plant tissues there were chromogens present which could be oxidized by oxidases into pigments. These pigments then served as hydrogen acceptors, i.e. in this case they bound the hydrogen of water, oxidation of the respiratory products being brought about by the oxygen. Generally, however, this formation of pigments would go unnoticed in the living tissues, as the pigment was again immediately reduced; it was only in necrobiosis, when the co-ordination of the various processes had been broken, that the pigment remained stable. PALLADIN held the view that all kinds of substances could play the part of chromogens, but his theory did not find much acceptance, it was not founded on facts and was too complicated.

(C) Chemistry of Respiration and Fermentation; Research after 1921

It was O. H. WARBURG [699] who in the year 1921 opened up entirely

new avenues in this respect. As so often happens in natural science, his work found its starting-point in one of those casual observations which may lead a brilliant investigator to far-reaching conclusions. In experiments concerning the respiration of the eggs of a sea-urchin, the carbon dioxide that was produced had to be determined by expelling it with the aid of tartaric acid. It was found that a solution of tartaric acid, which is in contact with living protoplasm, is oxidized by atmospheric oxygen. Hence the cell-content had to include a catalyst that oxidized the tartaric acid which would otherwise remain stable. This catalyst was found to be an iron compound which in the eggs of the sea-urchin is present in traces. This led WARBURG to wonder whether such iron compounds might have something to do with the physiological oxidation in the cell. It was found that not every iron compound had this particular action: it had to be a complex iron compound; and before long WARBURG could construct a model representing respiration with iron compounds by way of catalyst. The pre-requisite was that the model should be capable of carrying out, under physiological conditions of environment and temperature, the oxidations of substances which are present in the living organism. Moreover the process would have to be possible with the aid of atmospheric oxygen, i.e. without ozone or hydrogen peroxide.

If haematin, a component of the pigment of the red blood corpuscles, is charred in a retort from which air is excluded, the result is a charcoal containing nitrogen and iron, in which nitrogen is bound to iron. This charcoal is capable of carrying out the processes referred to: amino-acids dissolved in water will at 37° C yield an almost physiological oxidation when this blood charcoal is added; WARBURG's experiments showed for instance: leucine yields ammonia, carbon dioxide and valeraldehyde. An interesting point was that this reaction can be inhibited in several ways and that this can be done by substances which also inhibit the oxidation processes in the living organism. Narcotics behave in this manner because of their low surface tension and replace the amino acids at the surface layer of the charcoal particles upon which the oxidation occurs. Hydrocyanic acid also has an inhibitory effect on the oxidizing action, although it does not lower the surface tension. For this reason WARBURG assumed that hydrocyanic acid acts by combining with the active ferric atoms. With charcoal prepared from sugar and with pure silicic acid an adsorbant but no catalytic action results, whilst charring in the presence of iron and organically bound nitrogen did produce active charcoal.

These observations led WARBURG to the conclusion that respiration in the cell also took place on the surface of solid particles, and to prove this he cited the following: by freezing and subsequently thawing the red blood corpuscles of birds a liquid could be obtained in which floated the stromata of the erythrocytes. By centrifuging, these could be separated from the liquid and the carbon dioxide production would then be found to be associated with the erythrocytes and not with the liquid.

Hydrocyanic acid, hydrogensulfide and carbon monoxide have a reversible inhibitory effect on respiration and these substances likewise react with heavy metals, WARBURG now made inhibition of respiration by carbon-monoxide his starting-point for further research. It is common knowledge that carbon monoxide is highly toxic to man, for when inhaled, this gas forms a compound with haemoglobin, so that no oxy-haemoglobin arises; inhalation of air containing no more than a fraction of 1% carbon monoxide is fraught with serious danger. Carbon monoxide, moreover, affects the tissue respiration, in which it competes with oxygen.

It had long been known that combinations of carbon monoxide and iron-porphyrin are sensitive to light, and when it was found that the catalysis by heavy metals just referred to possesses the same sensitivity, WARBURG naturally assumed that the respiratory enzyme was a compound of iron and porphyrin.

WARBURG made a close study of this influence of light on the inhibitory action of carbon monoxide. It appears that the effect of illumination upon respiration inhibited by carbon monoxyde, which causes it to increase in intensity, is — as was already said above — similar to its effect on the action of a iron-prophyrin compound. Hence it may be concluded that respiration is a catalysis by heavy metals, in this instance by a compound of iron and porphyrin. At that time, the year 1928, compounds of this kind were already known, e.g. the so-called histohaemin of MAC MUNN [401], which D. KEILIN [317] later called cytochrome.

This sensitivity to illumination, which is specific for inhibition by carbon monoxide, was seized upon by WARBURG as a means of determining the absorption spectrum of the respiratory enzyme, since the effect produced by light must be due to the rays that are absorbed by this enzyme. It was found that under the influence of monochromatic light of varying wave-lengths the rate of absorption varied for different parts of the spectrum and it might be assumed that the action of light was proportional to the absorption by the respiratory enzyme; the absorption by other components can be disregarded, since these do not possess that specific sensitivity to light in these circumstances. In this way WARBURG found an absorption for the respiratory enzyme which greatly resembles that of an artificially prepared haemonicotine, or a haematine from the marine worm *Spirographis*. It is not, however, identical with any other natural haematin compound,

Incidently I would add that structurally these substances lie between the pigment of blood and chlorophyll. From this fact bold conclusions might be drawn to the effect that speaking phylogenetically, the respiratory enzyme is the fundamental substance from which protohaematin in the animals and chlorophyll in plants are derived.

Summarizing, it may be said that as early as 1928 WARBURG formulated a theory of which the essence is that the living cell contains an organic

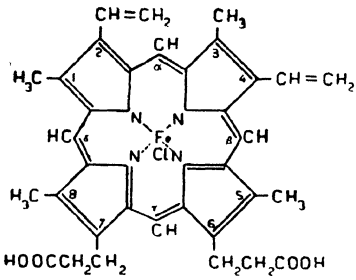


FIG. I. Protohaemin

iron compound of the phaeohaematin type, which activates atmospheric oxygen. It was based on the fact that respiration is inhibited by hydrocyanic acid and carbon monoxide and that the inhibition of the latter is sensitive to light.

Against this theory serious arguments were raised, e.g. that with this respiratory enzyme it was possible to oxidize amines but not monosaccharides; WARBURG's view concerning the oxidation of fructose was contested. Moreover this theory did not fit in with the hypothesis, referred above, that in both fermentation and respiration a zymase complex was active.

For these reasons we must now briefly consider the views of H. WIELAND [724]. He argues from totally different premises, namely the hydrogenation of organic substances by means of a catalysis by metals in which activation of hydrogen plays a part. WIELAND points out that the energy required for the dehydrogenation of one substance can be obtained by linking this reaction to the hydrogenation of another substance, the so-called acceptor. The point of attack of the catalysis, however, would then be the substance supplying the hydrogen, i.e. the hydrogen donor. As early as 1912 WIELAND discussed biological oxidation and argued that catalytic oxidation of ethyl alcohol to acetaldehyde and further to acetic acid may occur in the absence of molecular oxygen, provided only water and an H-acceptor, e.g. methylene blue, be present. These reactions are equilibrium reactions and thus reversible. As long as equilibrium has not been reached, the system remains capable of action; in other words, it is a redox system with a certain redox potential. Formation of acetic acid from acetaldehyd may occur in the presence of water by hydrate formation with $CH_3—C(OH)_2H$ as intermediate compound which can be dehydrogenated into acetic acid by means of methylene blue. We have to leave undecided what is the part played by the catalyst; it is possible that the H-ions are detached from the electronic field of the molecule by virtue of adsorptive powers.

WIELAND was supported by the physiologist T. THUNBERG [651] who studied the oxidation of succinic acid under influence of muscle tissue in an anaerobic environment. THUNBERG suggested the hypothesis that normal respiration is to be regarded as a chain of successive dehydrogenations, in which the free oxygen finally acts as hydrogen acceptor. Then the carbon dioxide would be, so to speak, the waste product of these dehydrogenations.

WIELAND's theory furthermore assumed that the oxygen which acted as acceptor was converted into hydrogen peroxide and that in the great majority of vegetable organisms this compound was directly broken down with the aid of catalase. This would explain the fact, which had been observed earlier, that the catalase content ran parallel with the intensity of oxidation.

Obviously a lively controversy was bound to arise between the supporters of WIELAND on the one hand and those of WARBURG on the other. WIELAND stressed the fact that although WARBURG was able to bring about oxidation of amino acids with his model of respiration, he failed to effect oxidation of glucose. He further argued that it sometimes happened that out of two stereo-isomers one. though not the other, could be fermented. (*Penicillium* breaks down dextro-rotatory tartaric acid much better than the laevo-rotatory tartaric acid). He put this as follows: "Die Zelle ist kein Ofen", the cell is no stove, WIELAND furthermore opposed the view that inhibition by hydrocyanic acid constituted evidence of a catalysis by heavy metals. According to him, when not oxygen but methylene blue acts as hydrogen acceptor, the inhibitory effect of hydrocyanic acid is almost nil, and he explains this by assuming that, when oxygen acts as acceptor hydrogen peroxide is produced which has to be decomposed by catalase. Hydrocyanic acid would have an inhibitory effect on the latter process. WARBURG, on the other hand postulated that in a catalysis by metals a totally different H-activation occurred, and said with some justification that WIELAND's explanation of the inhibition by hydrocyanic acid was sheer nonsense. In his publication on "Die Grundlagen der Wielandschen Atmungstheorie" WARBURG winds up by saying: "Da es keinen Versuch gibt, der die Theorie begründet und keine Konsequenz der Theorie, die zutrifft, so soll sie in der Folge nicht mehr berücksichtigt werden." (Since the theory is neither based on any experiment nor borne out by any actual observation, it should henceforth be left out of account").

But this was not really helpful, and various attempts were made e.g. by A. VON SZENT GYÖRGYI [640], A. J. KLUIJVER [331] and H. J. DONKER [141] — to reconcile the two theories. In his "Handbuch der Fermente", C. OPPENHEIMER [477] spoke of "eine einheitliche Deutung", (a central all-embracing explanation) and wanted to cancel thesis and antithesis in a higher unity in the manner of Hegelian logic.

KLUIJVER and DONKER pointed out that a similarity between respiration and fermentation could be traced in the following way. The facts which we have observed fit into the framework of WIELAND's concerning a gradual dehydrogenation and the assumption that in normal respiration decomposition of glucose begins in the same way is supported, moreover, by various facts. Why, then, is the ultimate outcome of the respiratory process different from that of the process of fermentation? The reason is that in normal respiration the oxygen in the air takes on the function of hydrogen acceptor, which is not

possible in anaerobic respiration. Later we shall again refer to the fact that fermentation is possible even in the presence of oxygen. KLUIJVER suggested the possibility that, in the final stage of the respiratory process, activation of oxygen might occur in the manner of WARBURG's respiratory enzyme and that the inhibitory action of hydrocyanic acid and carbon monoxide have a bearing on this part of the process.

At this point we have to consider yet a third line of approach. As stated earlier, in the year 1886 MAC MUNN [401] discovered a haematin compound in animal tissues; later KEILIN [317] also found this substanc in plants and gave it the name of cytochrome. In many cases it could be spectroscopically observed, and it was found that, in reduced condition, this cytochrome shows four absorption bands, which upon admission of oxygen, make way for two others. This is easily observable, for instance, in a yeast suspension, as is also its reversible character. According to KEILIN, one can thus visually observe, the respiration of yeast.

KEILIN later found that, instead of one, there are three cytochromes, of which cytochrome c in particular could be isolated out of yeast and proved to be a haematin compound, which as was found by THEORELL [648], could be oxidized in an acid solution with the aid of oxygen. This seemed to justify the assumption that this cytochrome was WARBURG's respiratory enzyme. But the latter strongly opposed this view on the ground that this cytochrome is not sensitive to the action of carbon monoxide. Moreover, this cytochrome c did not react with oxygen at the p_H of the cell. So that in these circumstances cytochrome c is not autoxidizable and there must be yet another catalyst in the cell.

This KEILIN countered by observing that hydrocyanic acid, carbon monoxide and hydrogen sulfide have an inhibitory effect upon indophenol oxidase, an enzyme found in all kinds of cells, which was later termed cytochrome oxidase.

Now what, is KEILIN's view of the action of the cytochromes in respiration? In the living cell, he says, oxidation of a cytochrome is effected by cytochrome oxidase. The oxidized cytochrome can be reduced in the absence of oxygen, provided that respiratory substances, such as succinic acid or lactic acid, are available, but this will take place only if a catalyst, capable of producing this reaction is also present, the so-called dehydrogenase. An interesting point is that this dehydrogenation is not inhibited by carbon monoxide or hydrocyanic acid, i.e. it is not a catalysis by heavy metals, but it is inhibited by narcotics. So the cytochromes are not themselves the enzymes of biological oxidation, but they are oxygen carriers which can play their part only in the presence of an oxidase, on the one hand, and of an dehydrogenase, on the other. One cytochrome carries the oxygen over to another.

At first WARBURG ignored KEILIN's views altogether, though he was later obliged to accept a good many of them.

KEILIN drew up the following scheme:

WARBURG SYSTEM	WIELAND SYSTEM
O_2 cytochrome oxidase cytochrome a, b, c,	dehydrogenase substrate
inhibition by HCN, CO, H_2S	inhibition by narcotics

At WARBURG's instigation, HAAS [220] ascertained which part of the respiration passes through the cytochrome system. The method followed consisted mainly in a study of the question as to which part of the reaction can be inhibited by hydrocyanic acid. It was found that in yeast practically the entire respiration passes via the cytochrome system and that this is also the case with acetic acid bacteria. It was also found there were organisms which did not behave in this way, e.g. lactic acid bacteria, a fact to which we shall refer again later.

WARBURG, NEGELEIN, [452] and HAAS drew the following picture of the respiration of acetic acid bacteria:

$$O_2 + \underset{1}{\text{ferro-ferri}}\ \underset{2}{\text{ferro-ferri}}\ \underset{3}{\text{ferro-ferri}}\ \underset{4}{\text{ferro-ferri}}\ \underset{5}{\text{ferro-ferri}} \ldots\ldots\ldots\ldots \text{alcohol}$$

According to them, this scheme is intended to indicate that the oxygen respiration of aerobic cells is a catalysis in which a chain of successive iron compounds is active by virtue of the valence alternation of their Fe-atoms. 1 is supposed to represent the oxygen-carrying enzyme; 3. 4. and 5 are the three components of the cytochrome system; 2 is an Fe-compound which is supposed to reduce 1 and to oxidize 3. The dots between the 5 th Fe-atom and the substrate represent enzymes which have not yet been investigated and which WARBURG c.s. call intermediate enzymes.

This is all very nicely put, but on close inspection we find that it is in reality tantamount to a partial capitulation to KEILIN on the one hand and to WIELAND on the other. For the section with the dots denotes nothing but the action of WIELAND's dehydrogenases, while 3, 4 and 5 denote acceptance of the significance of the cytochromes. 1 represents the action of WARBURG's respiratory enzyme, nowadays called cytochrome oxidase. 2 appears to be little more than a figment of the imagination. The reason why WARBURG c.s. assumed the existence of 2 lies in the absorption bands which may be observed in the different haematin compounds. In the acetic acid bacterium, *Acetobacter pasteurianum*, as well as in *Azotobacter*, they believed to be able to demonstrate the absorption bands not only of the respiratory enzyme, but also those of another haematin.

In a sense one may say that in that same year 1933 both WIELAND

and BERTHO [44] accepted the „einheitliche Deuting", so that agreement was finally obtained.

To revert once more to the „dotted" section one wonders on what grounds WARBURG came to the conclusion that his respiratory system does not act upon the substrate, in this instance glucose. This was due to his study of the respiration of the blood corpuscles of mammals. It was found that these show a much more intensive respiration when their environment contains methylene blue, while small concentrations of hydrocyanic acid do not have any inhibitory effect. At first WARBURG's opinion was that haemoglobin was converted by methylene blue into methaemoglobin which was subsequently reduced by the carbohydrate, but this assumption was incorrect; neither glucose nor phosphorylated glucose react with haemoglobin. Therefore WARBURG was forced to assume that catalysts as yet unknown played a part here. This was a concession to WIELAND, although WARBURG did not speak of activation of the hydrogen, but of activation of the substrate. WARBURG and CHRISTIAN [114] also found that the bright red liquid which is obtained by centrifuging autolysed blood corpuscles and which, on phosphorylated glucose being added, does not bind oxygen, will do so in the presence of methylene blue. Hence WARBURG had to give up his view that oxidation will only take place if solid particles are present: in this instance the process of oxidation took place in a clear colloidal solution. In this solution one or more enzymes or complex-enzymes must have been present; the addition of aluminum hydroxide rendered the autolysate inactive, the enzymes had been adsorbed on the aluminum. What were these adsorbed substances? They were found to show a marked resemblance to those of the zymase complex of yeast, which had been studied first by HARDEN [234] and YOUNG [748], later by EULER [163] and his pupils; EULER termed these substances apozymase and co-zymase. As was already said above is the former not dialysable and is destroyed at a temperature of 100° C; the latter is dialysable and can resist a temperature of 100° C.

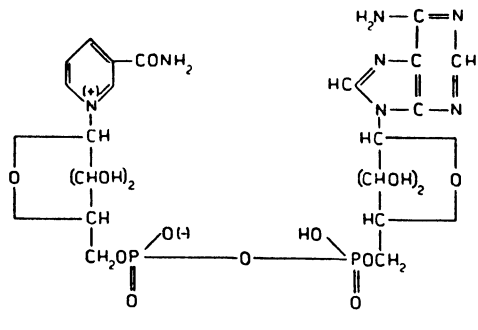


FIG. II. Cozymase

The way in which co-zymase was purified cannot be discussed here; though mention must be made of the fact that this co-zymase of HARDEN and YOUNG consists of the following components:

(1) adenine (aminopurine)
(2) nicotinic acid- amide
(3) ribose, a pentose
(4) phosphoric acid.

According to whether two or three molecules of phosphoric acid take part in the formation, we speak of di-or triphosphopyridine-nucleotide.

Thus it may be said that of this enzyme the so-called active group, the agon, is chemically full known. But as already said above this agon can become active only if it is bound to a specific protein, the pheron, which cannot stand heating to 100° C. It should be added, moreover, that in the binding of hydrogen the active group is the pyridine ring of nicotinic acid.

With the aid of this enzyme system it is possible to dehydrogenate, for instance, hexose-monophosphoric acid into phosphohexonic acid, in which case an aldehyde group becomes a carboxyl group with uptake of water.

$$Co \text{ (co-enzyme)} + RCHO + H_2O = CoH_2 + RCOOH$$

How does the process proceed? Only a small part of the substrate can be dehydrogenated, as the action must come to a standstill when the enzyme system, of which only a small quantity is available, has taken up all the hydrogen it can bind. For the process to be able to go further, the enzyme system has to be dehydrogenated again, i. e. it has to be deprived of its hydrogen with the aid of another system, the so-called yellow ferment or yellow enzyme. (flavin system).

In the year 1932 there appeared a publication by SZENT GYÖRGYI [640] and BANGA [28], in which they demonstrated that in the heart muscle an oxidation-reduction system is present which is rendered inactive by arsenic acid. In oxidized condition it is yellow, in reduced condition it is colourless, and the supposition was made that it played a part in the dehydrogenation of lactic acid in the muscles.

BERTHO [44] and GLÜCK [199] had studied the respiration of lactic acid bacteria; they stated it to be the first case in which respiration was found to be according to WIELAND's hypothesis.

A little later WARBURG and CHRISTIAN published an article on a new oxidation enzyme which according to them, was active in yeast and lactic acid bacteria and which appeared to be identical with that found by SZENT GYÖRGYI and BANGA. It could be reduced by phosphorylated glucose, though only in the presence of the so-called intermediate enzyme and the co-enzyme. Soon afterwards it was found that this yellow enzyme is of great importance in the respiration of various organisms. WARBURG held it responsible for that part of the respiration of lactic acid bacteria, which keeps going after addition of hydrocyanic acid, and this part is a very important one. The chemical constitution of this system was also ascertained; lactoflavin was found to be 6, 7, dimethyl-9, ribotylisoalloxazine.

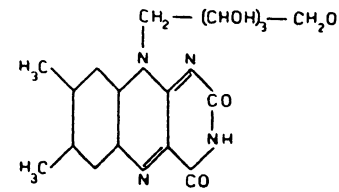


FIG. III. Lactoflavin

Here, again, activity is dependent upon combining with a specific protein. The H. appears to become bound to one of the nitrogen atoms, the value of which may alternate between 3 and 5.

How do we know that practically the whole of the respiratory process of lactic acid bacteria may pass through the yellow enzyme? In the answer to this question the so-called rate of alternation plays an important part; this is the factor which indicates how many times per minute the enzyme system can be oxidized and reduced again. In lactic acid bacteria the rate of alternation was found to be about 30, i.e. approximately the same figure as that obtained in a model experiment to test the oxidation of hexose-monophosphate. In other words, in lactic acid bacteria the enzyme is dehydrogenated with sufficient velocity to play its part which can be described as follows: the yellow enzyme withdraws hydrogen from the intermediate enzyme + co-enzyme and thus becomes itself hydrogenated. but is in turn dehydrogenated by the oxygen in the air; hydrogen peroxide being formed in the process.

In determining the rate of alternation in yeast, we find a value of approximately 19000, i.e., in this instance only a very small part (about 1%) of the respiration occurs in the same way as in lactic acid bacteria. This agrees with the fact that respiration is so strongly inhibited by hydrocyanic acid. In yeast the process is more complicated by the fact that the cytochrome system plays a part in it.

WARBURG assumed that the yellow enzyme in the cell can be oxidized by atmospheric oxygen in the same way as can be done in vitro. It may be questioned whether this assumption is correct. In the year 1937, THEORELL [648] made the important discovery that cytochrome c is also capable of effecting the oxidation of leucoflavin (hydrogenated flavin). Carrying out this dehydrogenation both in the presence and in the absence of cytochrome c and with varying oxygen tensions, he found that direct dehydrogenation was stronger as the oxygen tension was higher, whilst oxydation via the cytochrome was stronger as the oxygen tension was lower. An additional difference was that in direct oxydation (dehydrogenation) hydrogen peroxide was produced, which did not occur in dehydrogenation via the cytochrome. Hence the question arises: how high is the oxygen tension in the living cell? In the animal cell, according to THEORELL, this is so low that oxidation of the yellow enzyme will occur practically entirely via the cytochrome. THEORELL further contests the accuracy of WARBURG's statement, that the yellow enzyme is concerned only in that part of the respiration which remains after inhibition by hydrocyanic acid.

Assuming these views of THEORELL to be correct, we arrive finally at the following picture of the respiration of yeast and acetic acid bacteria, that of the higher plants will be dealt with later.

(1) phosphorylation of the glucose by the phosphatase;
(2) in contact with the system intermediate enzyme + co-enzyme the phosphorylated glucose is dehydrogenated by the carrying over of H via the pyridine ring;
(3) The hydrogenated co-enzyme is dehydrogenated with the aid

of the yellow enzyme, so that the co-enzyme becomes available again for further action;

(4) the hydrogenated yellow enzyme (leucoform) is once more dehydrogenated with the aid of cytochrome c which obtains its oxygen indirectly from the atmosphere, hence the hydrogenated flavine becomes available again;

(5) the cytochrome system is oxidized by means of a cytochrome oxidase, formerly termed WARBURG's respiratory enzyme.

In this way the carrying over of hydrogen and oxygen as the result of a series of co-ordinated enzyme reactions becomes clear, but the intermediate stages passed through by the substrate are still unknown to us; according to the description given above, oxidation did not go beyond hexonic acid. It is alleged by WARBURG and CHRISTIAN that by making use of the combination of the co-enzyme with another protein ,acting as pheron, they obtained a dehydrogenation far beyond that stage.

Alcoholic fermentation.

MEYERHOF [427], LOHMANN [390], PARNAS [496] and others studied the intermediate products between glucose and ethyl alcohol. Since the isolation of adenosine triphosphate (A.T.P.) from animal tissue, the part played

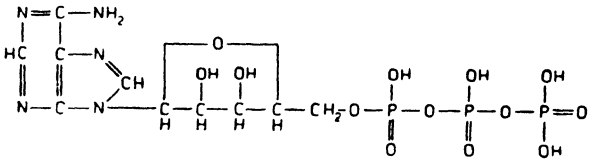


FIG. IV. Adenosine triphosphate

by this compound in the circulation of phosphoric acid in the living cell has been made clear.

This substance has the function of a phosphate donor. It looses one or two molecules of H_3PO_4 and is converted into adenosine diphosphate (A.D.P.), resp. into adenosine-monophosphate or adenylic acid.

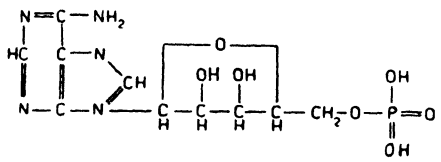


FIG. V. Adenylic acid = adenosine-monophosphate

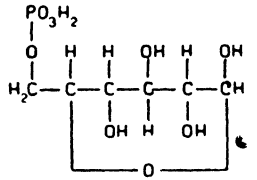


FIG. VI. Robinson ester

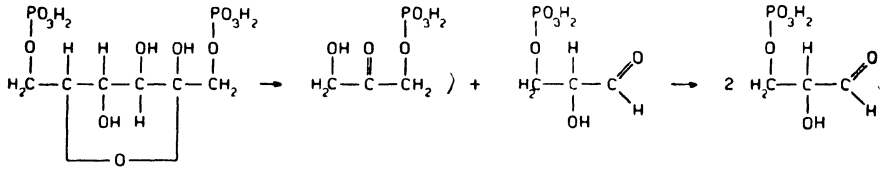
If glucose is phosphorylated no inorganic phosphate is bound but phosphoric acid can only be taken from the above mentioned A.T.P. At first one molecule with formation of hexosemonophosphate ester or Robison ester is bound.

Later a second molecule H_3PO_4 is bound by a slightly changed Robison ester. In this manner the above mentioned HARDEN-YOUNG ester (hexosediphosphate ester) is built up.

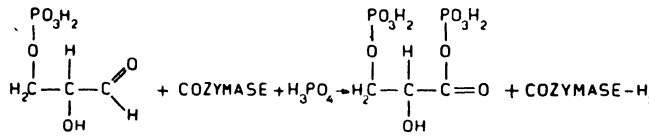
The balance equation is:

glucose + 2A.T.P. = Harden Young ester + 2A.D.P.

The Harden-Young ester is then broken down by the enzyme aldolase, a part of the zymase complex, into two triose-phosphate esters i.e. dihydroxyacetone-phosphate and glyceraldehyde phosphate. The former is then changed into the latter by the enzyme isomerase, which is also a part of the zymase complex.



Harden-Young ester Dihydroxyacetone-phosphate Glyceraldehyde-phosphat



Glyceraldehyde-3-phosphate Glyceric acid-1.3 phosphate (1.3-Phosphoglycericacid

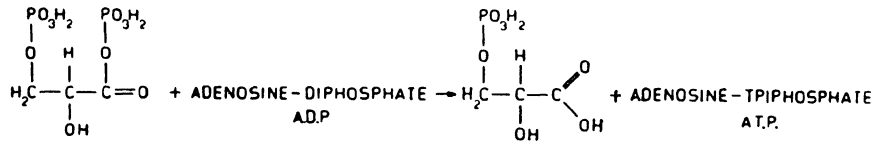


FIG. VII. — 1.3-Phosphoglyceric acid Glyceric acid-3-phosphate

As WARBURG maintains the next step is the transformation of glyceraldehyde-phosphate into the diphosphoric ester. Latter compound might be dehydrogenated into glyceric 1.3 phosphate, a substance isolated by NEGELEIN. It was once more WARBURG who proved that the apoferment (apo-enzyme) is active only if bound to co-zymase. The glyceric acid 1.3 phosphate can be split up spontaneously into 3-phosphoglyceric acid and H_3PO_4; it is also possible however that phosphoric acid is taken up by the above mentioned adenosine compound A.D.P., which changes into A.T.P.

Be that as it may, once formed glyceric acid 1.3 phosphate is via glyceric acid 3 phosphate changed into phosphopyruvic acid. The latter

substance yields its H_3PO_4 to adenylic acid whereby the formation of pyruvic acid is achieved at last.

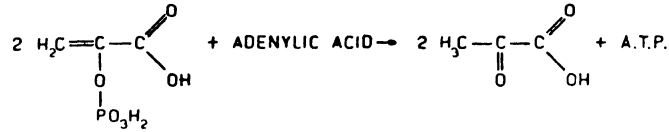


FIG. VIII. Phosphopyruvic acid Pyruvic acid

It is interesting, as MEYERHOF points out that this last reaction is not reversible, which renders the whole alcoholic fermentation process an irreversible one.

By maceration of yeast a sap can be obtained in which pyruvic acid is broken down into acetaldehyde and carbon dioxide, as was already mentioned above. This process is caused by carboxylase, whose co-enzyme, co-carboxylase has the following formula.

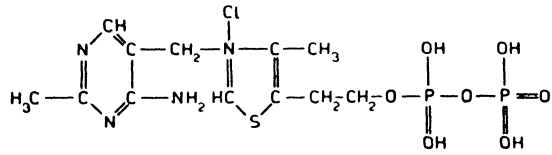


FIG. IX. co-carboxylase

Finally fermentation is completed through the action of the reducing fermentation enzyme called alcoholic-dehydrogenase by EULER, which reduces acetaldehyde to ethyl-alcohol. The prosthetic group of this enzyme is co-zymase, as in the oxidizing yeast enzyme, the protein carriers are different however.

One may legitimately ask what can be the physiological significance of all these complex reactions in the yeast cell? That it cannot consist in the formation of the final products, alcohol and carbon-dioxyde is obvious, because these are set free. Much more is to be said in favour of the hypothesis that in this manner the living yeast cell is able to derive the greatest benefit from the chemical energy bound in the substrate. This theory is corroborated by a phenomenon which is to be observed in animal tissue. In muscular contraction — a process in which the chemical energy bound in glycogen is used — nearly the same reactions occur as in alcohol fermentation. In the muscle the formed pyruvic acid is not decarboxylated but hydrogenated to lactic acid with the aid of co-zymase. Adenosine-triphosphate (A.T.P.) plays a part in the process and its splitting up into adenylic acid and phosphoric acid produces the chemical energy that enables the muscle to contract. In the phosphoric ester of the carbohydrates is less energy bound than in those of adenosin, 1,3 glyceric acid and pyruvic acid; in the former 2000-4000 cal. in the latter 8000-12000 cal.

The function of these reactions in fermentation must be as follows. Both yeast cell and muscular tissue try to raise the energy, binding the phosphate to the rest of the molecule as high as possible. At first, in the binding of carbohydrates this quantity is small but afterwards it is raised as soon as phosphoric acid is coupled to glyceric acid 1.3 phosphate (1.3 phosphoglyceric acid) and to phosphopyruvic acid. In the former case this is caused by a process of oxidation, in the latter by withdrawal of water. The latter is specially important because this withdrawal is wholly reversible, and the total energetic value of both compounds must be nearly equal. This implies that by withdrawal of water the distribution of intramolecular energy is changed and accumulation of energy appears in the phosphate bound. The way in which this transfer of energy takes place in yeast cells is still unknown; the muscular process has already been referred to.

Before we can summarize what has been achieved in this branch of natural science during the past fifty years, we shall have to assess the advancement of knowledge of respiration in the higher plants. Up till now we have only dealt with this process in yeast and bacteria, with regard to the higher plants I shall begin by discussing the respiration in the spadix of *Sauromatum*, an object specially studied by VAN HERK [253].

As we pointed out above, a greatly intensified respiration suddenly begins in the spadix of the Araceae at a given moment prior to pollination. It was found that in this process an enzyme-complex is active which is analogous to, though not identical with the zymase-complex. This exceptionally intensive respiration produces a rise in temperature in the spadix. Closer investigation has shown that this respiration takes place mainly in the peripheral parts of the cortex of the spadix. Translocation of the respiratory material seems to take place more centrally, and this material is accumulated in the form of cane sugar or in the form of starch, up to 28% of the dry weight. The respiration intensity rises in a very short time to maximally 30 times its original value. The process is set in motion by an activator which is produced in the region of the male flowers and transported to the appendix. The great respiration intensity only lasts a few hours, but during that period anywhere from 1/8 th to 1/3 rd of the available carbohydrates may disappear. Throughout this time the addition of glucose, hexose mono-or diphosphate fails to produce any increase of respiration, from which it might be concluded that the respiratory enzymes have become saturated Whether this view is quite correct, i.e. whether the velocity of the entire process is determined by the rate at which the enzymes can transmit the hydrogen and oxygen, is still uncertain, At a later stage the substrate concentration does constitute the limiting factor i. e. determines the velocity of the process.

When inquiring into the mechanism of this respiratory process, we must stress the fact that in this instance respiration does not appear to take place with the aid of the haematin system referred in connection

with yeast. For any haematin that may be present can be converted into a pyridine haemocompound, which in the presence of cytochrome has a characteristic spectrum observable by means of the spectral photometer. In this case there is no question of this compound however. Cytochrome oxidase is absent and the inhibitory effect of hydrocyanic acid is but slight. So here we find a marked difference between this respiratory process and that which occurs in animal tissue or in yeast and acetic acid bacteria.

An obvious question that arises is which enzymes are present in this instance. According to VAN HERK's observations they are: (1) hexose monophosphate dehydrogenase i.e. the intermediate enzyme with the co-dehydrogenase; (2) hexose diphosphate dehydrogenase; (3) an alcohol dehydrogenase. Co-zymase particularly is present in large quantity; approximately as much as in yeast and more than 100 times as much as is usually found in leaves. What is also remarkable is the proportionally large quantity of the flavine system; this amounts to about 10 times the average found by EULER c.s. in other parts of higher plants. In addition, catalase is present, so that practically all the enzymes referred to earlier are available, with the exception of cytochrome oxidase.

The significance of the flavine system can be ascertained by means of the rate of alternation which prior to the intensification of respiration is between 40 and 50, at the maximum of respiration approximately 140, and thereafter again 50. If the yellow enzyme was responsible for the entire quantity of oxygen used, the rate of alternation could not be much higher than 50, or in other words, at its maximum about 1/3 rd of the respiratory process takes place via the flavine system. Hence it must be assumed that the oxygen tension in the tissue is such that the flavine system is sufficiently autoxidizable. I should point out that in a flavine action of this kind, i.e. without cytochromes, which on the whole proceeds slowly, formation of organic acids occurs, and that this is also the case in the spadix of *Sauromatum*, as stated above.

Other investigators have made use of the fact that the inhibition of the haematin system by carbon monoxide can be partially overcome by means of illumination, a process which is reversible, as we have seen before. In this way the presence of a haematin system in pine needles and various other leaves was discovered by W. KEMPNER [319]; though in variegated leaves and in white flower petals this system seems to be lacking. P. M. MARSH [409] and D. R. GODDARD [200] found that in the root and the young leaves of *Daucus Carota* a cytochrome oxidase is responsible for the greater part of the respiration, but this is not so in the old leaves.

JAMES [294] and collaborators have given reasons for supposing that the ascorbic acid system may be active in the normal aerobic respiration of the barley plant. A link was established between this well investigated oxidation system and an important product of glycolysis viz. the above mentioned intermediate products.

Ascorbic acid is universally distributed in plant tissues. Oxydation of ascorbic acid to dehydroascorbic acid is reversible and SZENT GYÖRGII first suggested that it might behave as a redox body. JAMES and collaborators have made this assumption very probable.

Malic and citric acid as well as succinic, fumaric and isocitric acid have been observed in plant tissues, but cis-aconitic acid, which occurs in the modified KREBS cycle was absent. Up to the present no evidence has been obtained that these substances act catalytically in hydrogen transfer, and BENNET-Clark c.s. has found strong evidence to the contrary.

Although there is still a good deal to be investigated, I hope that with above I have succeeded in giving an indication of the general trend of the study of respiration during the past few decades. Nowadays we regard the respiratory process as gradual dehydrogenation of the substrate, which is effected with the aid of a very complicated system or systems of dehydrogenases and oxidases sometimes coupled with oxygen carriers. Each of these processes has been realised in vitro, so it is a case of aplasmatic metabolism. But what is the part played by the living protoplasm?

Before attempting to answer that question, we have to consider one more point, viz. the question as to what causes fermentation to arise in the metabolism of *Saccharomyces* even in the presence of oxygen. In *Saccharomyces cerevisiae*, beer yeast, not only dehydrogenases, but also the flavine system and cytochromes with cytochrome oxidase are present, so, given a sufficient quantity of oxygen to act as hydrogen acceptor, it might be expected that only respiration would occur.

In my opinion the first question is whether fermentation in an aerobic environment is in fact inhibited as compared with that in an anaerobic environment. After PASTEUR's work this question was also a point of controversy, and it was not until MEYERHOF's time that the technique had advanced sufficiently to enable reliable results to be obtained. MEYERHOF found that, when oxygen is admitted, fermentation sometimes declines considerably, though there are varieties, such as bottom yeast from breweries, in which, even with the best possible aeration, respiration does not account for more than 1% or 2% of the sugar utilized, whereas fermentation uses 98 to 99%.

MEYERHOF also investigated the influence of hydrocyanic acid, and hydrogen sulphide on fermentation. Under this influence the respiration which passes through the cytochrome system is inhibited and aerobic fermentation should rise to approximately the level attained in the absence of oxygen. This was contradicted by authors who worked with bottom yeast which at best has a very weak respiration so that any decrease thereof can have but little effect on fermentation.

J. C. HOOGERHEIDE [268], a pupil of A. J. KLUIJVER [331], devoted considerable research to this problem and arrived at the following conclusions: the fact that fermentation is maintained besides respir-

ation even when oxygen is admitted must be ascribed to a surplus of dehydrogenation products. With a high concentration of sugar, the velocity of both fermentation and respiration is independent of that concentration, the capacity of the redox enzyme system is pre-eminently the only determining, we can say, the limiting factor. If the concentration of sugar is low, respiration will be maintained if oxygen is available, though the intensity of fermentation will decrease, and it is not until fermentation has come to a standstill that respiration begins to decline. The same thing occurs when in an increasing concentration of iodo-acetic acid the action of the dehydrogenases is inhibited. The fact that in aerobic fermentation it is particularly the respiration which is inhibited by hydrocyanic acid, is due to the effect of this poison on the final links in the process, i.e. the cytochrome system which rests on catalysis by the heavy metal, iron. Respiration and fermentation are indissolubly linked together, but owing to the abundance of starting material and of reductases, the cytochrome system is incapable of oxidizing all the intermediate products and another substance steps in to act as hydrogen acceptor. In connection with NEUBERG's views, which we discussed above, there is every reason to assume that this acceptor is acetaldehyde which arises through decarboxylation of pyruvic acid. Since MEYERHOF's investigations 3-phosphoglyceric acid has been regarded as an intermediate stage between this pyruvic acid and phosphorylated glucose. The functions of adenosine phosphates in this process and the other intermediate products were mentioned above.

But this does not account for the hitherto unexplained fact that, when oxygen is admitted, fermentation is inhibited to a far greater extent than respiration is increased. In the view of some investigators the explanation might lie in the fact that the dehydrogenation system undergoes oxidative inactivation; this agrees with the assumption that every system works within a certain redox-potential range.

Let us suppose all the intermediate stages of fermentation and respiration to be known to us — which will certainly be possible in the near future — how, then, about our insight into these various processes? We know that in a co-ordinated system of oxido-reduction enzyme-complexes the substrate is gradually dehydrogenated and oxidized with the aid of water molecules or oxygen. We know the intermediate stages that are being passed through and assume that the redox potential determines which system shall come into action. Shall we then be able to say that the entire respiratory process has been physiologically explained? In my opinion the answer must be in the negative, despite the great progress made during the past fifty years. In the first place the power to produce the various dehydrogenases and oxidation systems at the right moment is still wholly unexplained and, moreover, the organisation of the entire system is more or less a mystery to us. The very co-ordination of all these physico-chemical processes in the living protoplasm constitutes the most essential element of life.

It is clear that an organisation of this kind is possible only if the living protoplasm possesses a certain structure; the cytological observations of W. SEIFRIZ [597] and others tend to confirm this idea. However, this subject lies without the scope of our book although in Chapter V a few words will be said about the structure of principal elements of protoplasm, the proteins.

(D) The Effect of Temperature on Respiration.

At the time when the Textbook was published it was known that with a rise in temperature, respiration increased in intensity, and it was believed that this rise continued until the vital limit had been reached. Hence it was thought at the end of the 19th century that, in contradistinction to other physiological processes, there was in this respect no question of a separate optimum and maximum since these were supposed to coincide. Careful research, a great deal of which was carried out in the laboratory of F. A. F. C. WENT [709] at Utrecht, has been devoted to this subject.

In the first place it was found that in respiration, as in many other physiological processes, the effect of temperature is governed by the enpirical rule formulated by VAN 'T HOFF [263] for the velocity of chemical processes at normal level of temperature, viz. with a rise in temperature of 10° C this velocity increases up to two or three times its original rate. ($Q_{10} = 2—3$)

Later we shall discuss more fully the question of limiting factors, i.e. that in a chain process, such as many physiological processes are, the slowest link determines the velocity of the entire process. In this instance it may be concluded that in respiration a chemical process determines the velocity of the whole chain of processes.

In accordance with this rule of VAN 'T HOFF then, the respiratory intensity rises until a certain temperature is reached which varies according to the object, but generally lies between 30 and 40° C; above this level some other factor begins to have an inhibitory effect. As a result of the two processes, the graph representing the effect of temperature on respiration does show an optimum. The shape of the curve varies according to the length of time during which the object is subjected to the action of the higher temperature. This matter was studied first by J. KUIJPER [357], later by D. S. FERNANDES [171], both of whom used germinating seeds in their investigations; the drawback of this particular object is, however, that it takes a long time before the inner part of the seed reaches the temperature of its environment. With smaller and finer objects, such as the mycelium of the fungus *Phycomyces nitens*, which S. R. VAN ASPEREN DE BOER [19] used in his investigation, this drawback is less pronounced and it is possible to arrive at more accurate conclusions. There again, the connection between temperature and respiration is represented by an optimum-

curve, but the optimum lies so close to the maximum, whether the production of carbon dioxide or the absorption of oxygen be taken as the gauge of intensity, that it is not surprising that formerly optimum and maximum were believed to coincide in this case.

A part of the graph is a straight line and WASSINK [701] explains this by assuming that the quantity of respiration material is the limiting factor here.

With a fall in temperature a decrease of respiratory intensity coincides in accordance with the rule of VAN 'T HOFF; the minimum approximately coincides with the lowest temperature the plant can tolerate, at least in those objects which are intensely alive and have a large water content. In objects that have a low water content and are in a state of rest, e.g. dry seeds or spores, respiration is so extremely slight that it is impossible to measure the effect of temperature, particulary a temperature below freezing point which these objects are able to withstand. Later I shall return to this question again, when dealing with the subject of refrigeration and death due to freezing.

(E) The Effect of Light on Respiration.

An important question is whether there is any evidence of an effect of illumination on respiration. With plants containing chlorophyll it is, of course more difficult to answer that question. A century ago it even used to be doubted whether there was any respiration at all in green parts of plants that are exposed to light. The intensity of carbon dioxide absorption is generally so much greater that respiration in the green, illuminated parts seems to be imperceptible. Yet the fact that there is a so-called compensation point i.e. the point at which neither carbon dioxide nor oxygen is given off by the green plant and where respiration and photosynthesis balance each other, is a clear indication that respiration does take place in illuminated green parts. According to the nature of the plant, whether it is a shadow-plant or a sunplant, this compensation point is reached in weaker or stronger illumination.

Moreover, by means of a weak narcotic it is possible to inhibit photosynthesis almost completely, whereas respiration is inhibited far less and remains clearly perceptible. It is, furthermore, a well-known fact that protoplasmic streaming occurs only in the cells of tissues where respiration takes place, and in various objects it can be easily observed that cells containing chlorophyll do have this streaming whilst under the influence of illumination.

So the question should, instead, be read as follows: does or does not illumination cause any change in the respiratory intensity? In green Algae F. VAN DER PAAUW [441] observed that during the period of darkness immediately following illumination, respiration is more intense than it was just prior to the illumination. In water plants, such as *Elodea* and *Potamogeton*, something of the kind was also observed by F. GESSNER

[144]. The explanation might be thought to be simply this, that through the proceeding illumination and photosynthesis respiratory material had been formed, but this does not explain GESSNER's experiments, in which the rise in respiratory intensity also occurs when illumination takes place by means of a mercury vapour lamp of which the visible part of the spectrum is absorbed by filters, so that the ultra-violet end remains and no photosynthesis occurs. The explanation of this phenomenon has not, so far, been elucidated; to speak of a stimulation by light does not give a real explanation: Discussion of the question whether illumination has any effect on the respiration of non-green plants, as well as a discussion of the parallelism observed by SPOEHR [618] and MAC GEE [399] between respiration and photosynthesis would lead us too far.

(F) The Effect of Environment on Respiration.

The textbook by DE VRIES already mentions that the carbon dioxide production of germinating seeds of *Vicia Faba*, the broad bean, is approximately as great in atmospheric air as it is in an anaerobic environment, at least during the first hours. In this instance anaerobic respiration is equal to the normal respiration.

In those days it was thought possible to alter the tension of oxygen within wide limits, for instance from 1/20th to 1 atmosphere, without causing any detrimental effects and without any deviation of the respiratory quotient from its normal value of, approximately, 1. Experiments made during the past decades have, however, shown that this does not hold good generally. ELSA REUHL [539] working in the laboratory of L. G. M. BAAS BECKING [35] found that the minimal tension of oxygen to enable a normal quantity of oxygen to be used, varies according to the object and the stage of germination. In wheat this minimal tension of oxygen is, first, 15%, later 20%; in buckwheat it is first 3%, later 11%.

Experiments with ripening apples carried out by F. V. BLACKMAN [52] and his pupils also produced quite different results. When the apples were taken out of atmospheric air into pure nitrogen, carbon dioxide production would rise rapidly up to 150% of its former value, thereafter it would begin to fall slowly. When they were moved out of the nitrogenous atmosphere into atmospheric air, the reverse was observed.

If it is assumed that in anaerobic respiration the same process takes place as in alcoholic fermentation, an assumption already discussed above, it might be expected that, with the removal out of atmospheric air into pure nitrogen, a decrease rather than an increase in respiration would result. For in fermentation one molecule of glucose yields two molecules of carbon dioxide while in normal respiration the oxidative decomposition of one molecule of glucose yields six molecules of carbon dioxide.

BLACKMAN tried to explain this observations by assuming that, in

atmospheric air, respiration is 4 or 5 times more intensive than the carbon dioxide production might lead one to expect; out of every 4 or 5 carbon atoms, which are subject to glycolysis, only one would be oxidized into carbon dioxide, and with the aid of the energy thus obtained the remainder would then yield glucose again by virtue of resynthesis. This fits in with the theory formulated by MEYERHOF concerning the process that takes place in the muscles. Such resynthesis has been argued also in other instances in plant physiology.

The tension of carbon dioxide in the atmosphere also influences the respiratory intensity; a high concentration of carbon dioxide in the atmosphere inhibits respiration; this had already been discovered by DE SAUSSURE [444]. In the beginning of this century T. KIDD [322] was able to establish this more exactly; for instance, in a gas mixture containing 20% carbon dioxide, the rest being of atmospheric composition, the respiratory intensity of germinating mustard (*Sinapis alba*) may decrease as much as 30%.

The question whether in parts of plants which live in atmospheric air, anaerobic respiration may occur besides aerobic respiration, as a result of low intercellular oxygen content, has been answered variously, particularly in regard to fleshy tissues.

In the past century H. DEVAUX [136] observed a practically normal oxygen content in intercellular spaces of fleshy tissues of fruits. Later investigators doubted or denied the accuracy of this observation on the basis of their own experiments. But a recent investigation, carried out with the necessary critical precautions in my laboratory by A. GORTER [205] and WILLY NADORT [448] has proved the accuracy of the results obtained by DEVAUX with potatoes and similar objects.

In other instances, particularly in seeds with a not very permeable seedcoat, the diffusion of gas may be greatly obstructed. For instance, in seeds of *Pisum sativum*, which had been stripped of their seedcoats G. FRIETINGER [187] found a much higher oxygen tension in the intercellular spaces than in those with seedcoats, whilst carbon dioxide production was approximately equal. The respiratory quotient of the seeds $\frac{CO_2}{O_2}$ in their seedcoats was over 3. One might be inclined to explain this by the assumption that in this instance also anaerobic respiration occurs. However this result was proved to be not necessarily due to a low pressure of oxygen by the recent investigations of W. RUHLAND [560], ULLRICH [664] and RAMSHORN [527], who found very high values for the respiratory quotients in growing points of various objects, e.g. a value of 6—7; but these values were observed in an atmosphere of pure oxygen, while to all appearances there was nothing to obstruct the diffusion of gas. The presence of alcohol in the cambium tissue of lime tree and lilac, which these investigators observed, is regarded by them as an additional indication that in these tissues anaerobic respiration does occur, though its cause is still obscure.

As already stated, the respiratory intensity is to a great extent dependent upon the quantity of protoplasm contained in the cells of a tissue. After the investigations of W. PALLADIN [494] in this regard it might even be assumed to be proportional to the quantity of nucleoproteins, i.e. to the quantity of nuclear substance, though this has not been strictly proved.

It is obvious that respiration may vary considerably in different tissues and that it is relatively stronger in young leaves than in old ones. in flowers than in full grown leaves, more intense in stamens than in petals. It would lead us too far, though, to discuss this in detail, the more so as it would not open up any new point of view. I would point out only that, if a plant is wounded, a reaction will set in, which is accompanied by the formation of wound tissue (callus) and in which invariably an intensification of respiration is observed. In potatoes that are being cut, for instance, respiration may become 10 times more intense, a phenomenon in which, no doubt, the greatly facilitated access of air plays a part. It is curious that there is also a small, though measurable, rise in temperature. This was measured by means of a thermo-electric method by the Swiss botanist E. GÄUMANN [191] and also by F. C. STEWARD [629]. In potato tubers a rise of 0,08° C. was found.

(G) Bio-luminescence.

The phenomenon of the emission of light by some organisms, the so called bio-luminescence, is also closely connected with respiration and may in a sense be regarded as a side issue.

Since the 17th century, when the physicist R. BOYLE [68] discovered that for meat to be luminescent the presence of air is required, the obvious assumption after the discovery of oxygen by PRIESTLEY [520] was that it must be this substance in particular which is essential to the emission of light.

Later it was found that, besides some luminescent animals, such as the glow-worm *Lampyris* and the stone-borer *Pholas dactylus*, there are also various bacteria which emit light; these have been grouped by BEYERINCK [48] under the genus *Photobacterium*. Such bacteria are responsible for the luminescence of meat, referred to above. In addition, there are also some fungi that are luminescent e. g. *Armillaria mellea*, whilst the phosphorescence of the sea along the Dutch coast is caused by the Flagellate *Noctiluca miliaris*.

At the end of the past century and the beginning of the present one R. DUBOIS [147] made some interesting observations in connection with the stoneborer. He found that a certain substance in the animal, viz. luciferine, is oxidized under the action of the enzyme luciferase, which is also present, in which process light is emitted, and that this reaction could also be realised in vitro. Later E. N. HARVEY [243] succeeded in demonstrating the same process in the Crustacean *Cypridina Hil-*

gendorfii. This reaction in vitro has not been proved possible in vegetable organisms, since the substances could not yet be isolated, but in view of the investigations by F. H. JOHNSON [300] it appears that in this instance also a similar process may be assumed.

Under the influence of more recent experiments and of modern views in respect of carbon dioxide absorption in photosynthesis the methods of flashing light and inhibition by hydrocyanic acid have been applied; we shall revert to this methods later in extenso when discussing the subject of photosynthesis. K. L. VAN SCHOUWENBURG [585] came to the conclusion that the emission of light is dependent upon that part of the respiration which is but little sensitive to the action of hydrocyanic acid, so that the haematin system probably does not play any part in bio-luminescence.

The influence of carbon monoxide on bio-luminescence appears to be of a rather more complicate nature, since with a low $\frac{CO}{O_2}$ quotient luminescence is stimulated whereas with a higher quotient it is inhibited. As stated above, carbon monoxide invariably has an inhibitory effect on respiration.

Pursuing HARVEY's line of thought, JOHNSON, EYMERS [167], VAN SCHOUWENBURG and A. VAN DER BURG [93] have drawn the following picture of the course of events:

$$L + XH_2 \rightarrow LH_2 + X$$
$$A + LH_2 \rightarrow ALH_2$$
$$2\,ALH_2 + O_2 \rightarrow 2\,A + 2L' + 2\,H_2O + h\nu$$

in which L represents oxyluciferine, LH_2 luciferine, L' the irreversible oxidation product of L which arises in the oxidation connected with the emission of light. Investigations concerning the chemical composition of luciferine, are still in progress, so they cannot be dealt with here.

(H) Conversion of Energy.

We must briefly discuss the conversions of energy that take place in the living plant. The subject has some connection with respiration, it can be best dealt with here.

In the year 1914 LUCY C. DOYER [145] studied these conversions in germinating wheat-grains in the laboratory of F. A. F. C. WENT [799], thus continuing the work done by H. RODEWALD [547] in the last century. During the first seven days of germination, the daily loss of energy was gauged by the decrease in the combustion-heat. This decrease is becoming greater gradually, while the amount of energy which was imparted to the environment in the form of heat, was invariably less than the said decrease.

About 20 years afterwards, L. ALGERA [3] carried out energy measure-

ments on *Aspergillus niger* in the laboratory of W. H. ARISZ [13] at Groningen with the aid of an automatic micro-compensation calorimeter.

In a living organism processes take place, in which a part of the food substances absorbed, is used to synthesize the protoplasm and the remainder is broken down in the respiratory dissimilation process. At the same time chemical, osmotic and electric potentials are set up, as was clearly elucidated by PFEFFER and later by NATHANSOHN [450]. All these processes entail the development of heat, the total of which was measured in these investigations, but it is difficult to ascertain the contributions of each of these processes apart. Whereas in a non-growing organism all the energy of the food absorbed is converted into heat, a fact which was established for animals by M. RUBNER [557] and for fruits and seeds by RODEWALD, this is not so in a growing organism, since an increase of the chemical, osmotic, and electric potentials is inevitable.

E. F. TERROINE [647] and R. WURMSER [746] used *Aspergillus niger* to determine the so-called growth -yield, i. e. the energy of the mycelium formed, divided by the difference between the energy of the used food substances and the maintainance energy. By maintainance energy is meant the energy required for maintaining the existing potentials.

For this actual growth-yield they found a value of approximately 72%, which shows marked difference from the value arrived at by V. HENRI [250] in his calculations of the growth -yield of nitrifying and sulphur bacteria. However ALGERA confirming the conclusions of M. MOLLIARD [438] found an actual growth-yield in *Aspergillus niger* of 94—97%; the method used by TERROINE and WURMSER is probably to be regarded as incorrect.

CHAPTER III

IMBIBITION AND DIFFUSION (PERMEABILITY)

(A) Imbibition.

The proces known as imbibition can be dealt with very briefly. Ever since the natural sciences began to develop, the subject of imbibition has on the one hand been treated as part of colloid -chemistry, while on the other hand, recent research tends to stress the structural aspect. Hence the subject is discussed in the section dealing with the structure of cell-wall and protoplasm, which is now regarded as coming within the scope of cytology.

Nevertheless, the imbibition-pressure of the protoplasm, remains of importance to physiology and will be referred to again later. The swelling of the cell-walls is also of importance in regard to some of the plant movements and will be mentioned in the chapter dealing with these movements.

(B) Permeability and Semi-permeability.

This part is devoted to the subject of diffusion, a branch of plant physiology under which DE VRIES includes permeability and semipermeability. These processes have been the subject of much controversy in physiology ever since the year 1895.

In view of the fact that it forms a chapter of plant physiology to which HUGO DE VRIES contributed fundamental work, this part of the textbook gives a good picture of the state of knowledge reached by the year 1895. Moreover, the researches of de Vries and Pfeffer [444] provided a solid foundation of factual material on which most of the recent superstructure of hypotheses has been built up.

The facts are so generally known that I need not deal with them in detail. Protoplasm, particularly the tonoplast, i. e. the boundary layer between protoplasm and vacuole, is semi-permeable, i. e. it permits water to pass rapidly though permeation of the majority of dissolved substances is almost completely obstructed. Hence, when water is present,

the protoplasm will swell, but the volume of the vacuole will also be increased through absorption of water, since it contains dissolved substances, viz. salts and sugars and has a so-called osmotic value. The surrounding cellwall thus becomes distended and, in resisting this enlargement by virtue of its tensile strenght, attains the rigidity and the state of tension which de Vries calls turgor. Hence the absorption of water will continue only until the tensile stress in the cell-wall balances the osmotic pressure of the cell content. By gradual dilution of a hypertonic solution de Vries was able to determine the concentration of the solution which is isotonic with the vacuolar sap. In the words of de Vries the conditions for the development of considerable tension between cell-wall and cell-content are the following:
(1) The presence of water-attracting substances in the cell-sap;
(2) great elasticity and resilience in the cell-wall;
(3) the protoplasm (tonoplast) must be alive;
(4) the presence of water in the environment.

Now as to the trend of subsequent research. For a number of years it was particularly the question of the real nature of semi-permeability, on which attention was focused. The laws of osmosis, on account of which osmotic pressure can be compared with gas pressure, had been clearly explained by PFEFFER. Owing to the work done by HUGO DE VRIES plasmolysis and the conclusions which can be drawn from plasmolytic observations had become generally accepted bases of physiology, on which VAN 'T HOFF [263] and ARRHENIUS founded their physico-chemical views, but the real nature of semi-permeability was still entirely unexplained.

HUGO DE VRIES hardly occupied himself with this problem: he was aware that at death of the protoplast, semi-permeability changes into almost complete permeability; he knew that plasmolysis is reversible in glycerol, a fact which was explained in this way that the tonoplast must to some extent, be permeable at least to this substance. He and his pupils also studied the permeability of urea and potassium nitrate.

In the year 1899, O. OVERTON [489] began a series of investigations concerning the permeability of the boundary layer of the proplast, to organic substances in particular. Whilst sugars and sugar-alcohols with 6 carbon atoms permeate extremely slowly, glycerol with 3 C-atoms permeates more rapidly and glycol, with 2 C-atoms permeates more rapidly still. It was the general opinion in those days that this was proved by the following facts: cells of *Spirogyra*, when in contact with hypertonic solutions of urea become deplasmolysed in the course of some hours; when in contact with glycerol, this takes place in two hours, and with glycol in a few minutes. Primary alcohols, aldehydes, mono-di-and trihalogens permeate with the utmost velocity, almost spontaneously. That rapid penetration into the cell by a substance such as ethyl alcohol is NOT due to the killing of the protoplasm

was proved by the experiments of W. OSTERHOUT [482] in his study of plasmolysis in a mixture of glucose and ethyl alcohol.

OVERTON's conclusion was that especially those substances will permeate which are soluble in lipoids, e. g. cholesterol, from which he concluded that the boundary layer of the protoplasm consisted of lipoids. According to OVERTON, the velocity with which substances that are soluble in lipoids are absorbed depends upon the proportion in which they are divided between water and oil; the more they are soluble in oil, the greater the velocity of their permeation.

This lipoid theory of OVERTON, which is really no more than an elaboration of the views of L'HERMITE and W. NERNST [455], is sometimes called the solution-theory.

OVERTON's theory soon became a point of controversy: pharmacists, physicians, physiologists were all greatly interested in this problem and the arguments for and against it were being debated everywhere. Serious arguments were raised against a theory which left totally unexplained penetration into the cell by essential substances, such as inorganic salts. Moreover, penetration by organic dyes, which RUHLAND [560] -following the lead of his teacher PFEFFER — regarded as a criterion of permeability, was found to fit in with OVERTON's theory only in part. The permeability to sugars, which appeared to follow from the observations formerly made by J. A. BÖHM [55] and J. VON HANSTEIN [233] concerning *Lemna* plants, which rapidly formed starch when placed in solutions of sugars, constituted another argument against it.

The conflicting evidence mentioned above, viz. that poisonous substances permeated rapidly, while essential substances did not so at all, or only slowly, led A. NATHANSOHN [450] to his so-called mosaic theory in the year 1904. This was a compromise; according to this theory, the boundary layer of the protoplasm was a mosaic, formed by lipoid particles dissolved in an oil phase and protein particles dissolved in a water phase; in other words, a hydrophilic protein phase beside a lipophilic phase. The lipophilic phase would account for the facts observed by OVERTON, while the hydrophilic protein phase would be responsible for the permeation of sugars and salts.

Permeability to the latter was a process which could be regulated by the living protoplasm, though permeability to the former was not. In colloidchemical terminology, the boundary was supposed to form a polyphasic emulsoid system.

Whilst the solution theory of NERNST had given rise to OVERTON's theory, the so-called sieve theory of M. TRAUBE [656] likewise found supporters among physiologists. TRAUBE compared the semi-permeable boundary layer to a sieve which only permits particles of a certain size to pass through. E. BECHHOLD [34] constructed gelatine membranes of varying permeability, which could serve as ultra-filters and compared the boundary layer of protoplasma with those (ultrafilter theory). In this connection the investigations of RUHLAND in particular came to

he fore: he tried to borrow arguments from the absorption or non-absorption of organic dyes which, he thought, was dependent upon the size of the particles. This led to endless discussions: repeatedly substances were found which appeared to confirm the sieve-theory until someone else would come along with fresh objects that did not fit in with it. In the year 1925, RUHLAND believed that in the sulphur bacterium, *Beggiatoa mirabilis*, he had found an object which completely bore out with the sieve-theory; the larger the molecules, the greater their difficulty in permeating. But in this instance, again, various objections can be raised. H. FISCHER [176], moreover, correctly pointed out that while a sieve-theory would account for the observation that small molecules do permeate and large ones do not, it does not explain why the velocity of the process should be inversely proportional to the molecular volumina.

Besides these theories — to which yet others may have to be added, as we shall find later — some of the numerous investigations on the subject must be mentioned, in particular those which furnish evidence of the complexity of the problem of permeability and its intimate connection with all vital phenomena. First of all we shall have to discuss the experiments on *Rhoeo discolor* which were carried out with extreme precision by H. FITTING [178]. He noted, for instance the time required for penetration by the plasmolyticum and found that permeability becomes greatly changed under the influence of the penetrating substance; during the first few minutes penetration takes place much more rapidly than afterwards. When FITTING placed the tissues first in an environment which is hypotonic, i. e. which does not plasmolyse, maximum plasmolysis was attained much later than when the cell was placed immediately in a hypertonic solution which does plasmolyse.

It is obvious that the velocity with which plasmolysis sets in must be dependent upon the velocity of permeation by the water molecules; the velocity of deplasmolysis, on the other hand, is dependent upon the rate at which the molecules of the plasmolyticum pass the protoplasm. In any case, therefore, the velocity of permeation by the water molecules must have become less in these tests.

In FITTING's research it also becomes evident that it is a question not of permeability of undissociated salts, but of permeability of ions, and that this is governed entirely by their valence, i.e. the electric charge of the ion. Monovalent ions permeate rather rapidly, those which are bivalent permeate much more slowly, and those which are trivalent hardly do so at all. Investigations by A. NATHANSOHN and later by W. STILES [630] GLADYS REDFERN [533] and others showed that anions and cations of a dissociated salt are not absorbed to an equal extent, a fact which will be referred to again later.

Here attention must be drawn to the phenomenon termed antagonism of ions; in biology this was studied first by the American animal physiologist JACQUES LOEB [386]. In common parlance this term antagonism of ions means that one ion obstructs or cancels the action of another ion,

generally one of a different valence. A correct proportion of antagonistic ions yields the so-called balanced solutions.

In botany it was OSTERHOUT [482] who studied this antagonism in connection with permeability. He found that, for instance, the calcium ion with a valence of two is an antagonist of the monovalent potassium or sodium ion. By mixing a solution of NaCl with a solution of $CaCl_2$, neither of which, when taken separately, has a plasmolytic effect on the cells of a certain filament of *Spirogyra*, a solution was obtained which does plasmolyse, provided that it is a mixture of 10 part NaCl to 1 part $CaCl_2$. The explanation might lie in the fact that small concentrations of the latter substance decrease permeability to a far greater extent than it is enhanced by a much greater concentration of NaCl.

OSTERHOUT formulated a so-called dynamical theory of antagonism, based on the assumption that the electric resistance of living tissue is a measure of antagonism. Because of its highly speculative character I shall not go further into this theory; rather will I consider the question of ionic antagonism in connection with the colloid-chemical views of W. C. BUNGENBERG DE JONG [92].

Before doing so, we must first discuss some investigations along the same lines as those of FITTING, viz. the experiments by the Finnish physiologist R, COLLANDER [124] and his pupils, who also attach great importance to the time factor in plasmolysis. Assuming that the plasmolytic end-concentration of sucrose = c and that of urea = c′, then, according to COLLANDER, the quotient $\frac{c'}{c} = \omega$, i. e., the osmotic coefficient of urea at that particular moment. If this ω is used as the ordinate and the time as the absciss, a certain graph will be obtained for each substance examined by determining these values at various moments. The line first ascends, but then comes a downward curve which is most likely attributable to permeation by the plasmolyticum. If for methyl urea, for instance, $\omega = 0{,}8$ after one hour, it may be assumed that this is the result of the fact that so much methyl urea has penetrated into the cell that its molar concentration is $\frac{1}{0{,}8} = 1{,}25$ times as great as that of sucrose. The velocity with which ω goes down, i.e. the size of the angle formed by the line with the horizontal axis, will then be the measure of the velocity of permeation.

By working along these lines, COLLANDER arrived at the same conclusion as OVERTON, viz. that accumulation of OH groups in the molecule diminishes its permeability. Replacement of H atoms by CH_3 again enhances permeability; in other words, there is parallelism with solubility in lipoids; there is also parallelism between the degree of permeability and the surface tension. COLLANDER and his pupils also found an effect of the molarvolume, the largest and smallest molecules permeate slower, respectively quicker than might be expected from their solubility in lipoids. This method is open, however, to the same

objection as that which may be raised against FITTING's method, viz. that permeability is measured only of protoplasm which was previously in contact with the substance in question.

Other physiologists point out that, though on the one hand the ultra-filter theory does not hold good and on the other, solubility is an extremely important criterion, it must nevertheless be said that substances with small molecules, such as water, urea and methylurea, definitely do penetrate more rapidly than can be reconciled with their solubility in ether. For this reason they refer to a lipoid-filter theory; in a sense this reminds one of NATHANSOHN's mosaic theory which could also be regarded as a kind of compromise.

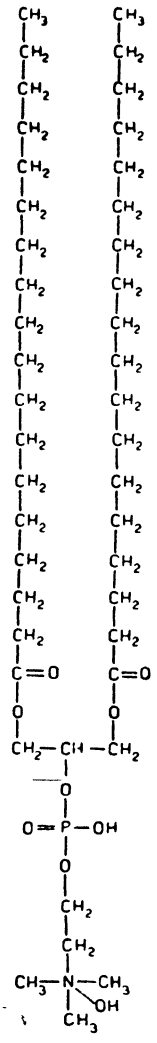


FIG. X. β Lecithin (ester of stearic acid)

Now we come to the colloid-chemical views of BUNGENBERG DE JONG and his pupils. Starting with the idea that the semi-permeable boundary layer has only molecular dimensions, this school tries to construct model films, the properties of which are as near an imitation as possible of these of the boundary layer of the living protoplasm. It would lead us too far into the realm of colloid-chemistry if we were to discuss this subject in extenso; it is possible only to mention some of the main points.

In this connection the question of the antagonism of ions, i.e. how one ion may modify the permeability for another ion, plays an important part; the permeability of the substances has also to be taken into account.

A more or less satisfactory picture is given by the double film of phosphatids schematically reproduced below, with a lipophilic layer of lecithin on the outside and a hydrophilic layer in between. The hydrophobic carbo-hydrogen chains of the phosphatids are turned outwards, while between fat molecules may still be present.

In a structure of this kind the positive and negative charges would keep the whole in a stable position.

According to BUNGENBERG DE JONG, a plasma membrane of this kind will show marked similarity to the boundary layer of the living protoplasm, both in regard to the organic substances referred to above and to inorganic substances, in this instance the ions and their antagonism. Without extensive colloid-chemical observations it is, however, impossible to explain this in full.

Some investigations must be mentioned, as a result of which our views in respect of the permeability of living protoplasm have become

modified in the course of the past 25 years. They concern the question as to whether there may be important variations of permeability, both of one individual cell with respect to another and of one and the same cell under different conditions.

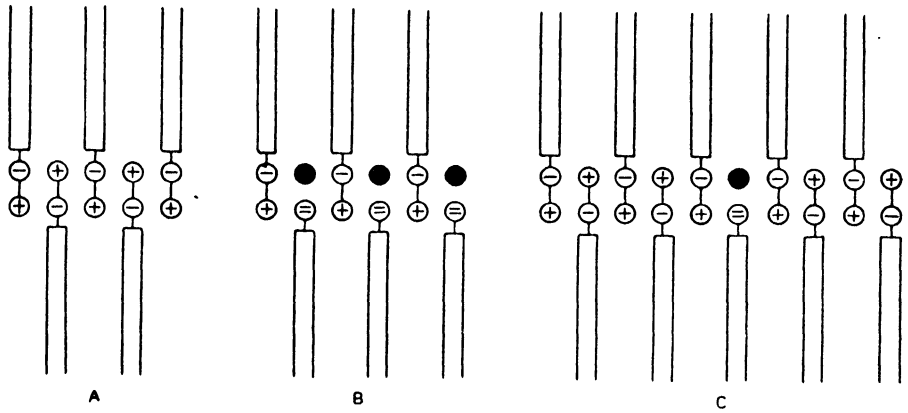


FIG. XI. C Transition between uni-tricomplextype plasmamembrane (A-B)
● bivalent calcium-ion
resp two monovalent potassium-ions

The former point was studied mainly by K. HÖFLER [262] and his pupils, who employed the usual method of plasmolysis and even more frequently the so-called plasmometric method. It would take too long to discuss the second method at length; briefly, it amounts to this that the volumina of cells of regular (cylindrical) shape and possessing a thin, parietal cytoplasm, are determined in hypertonic solutions of varying concentration, the result being used in the calculation of the change in degree of plasmolysis per unit of time.

By virtue of this method, HÖFLER arrived at the conclusion that permeability to water, for instance, varied greatly in different objects examined by him. In cells of *Zygnema Velox*, which belongs to the Conjugatae, the velocity is about 10 times greater than in cells from the leaf tissue of *Vallisneria* and approximately 100 times greater than in cells of *Salvinia*,

Investigations by Is. DE HAAN [219] show that this permeability to water is governed by a process of swelling in the protoplast. The detrimental action of both plasmolysis and deplasmolysis — like the detrimental action of distilled water — seems to be caused by a too rapid swelling of the protoplast.

According to HÖFLER and HUBER [282] some cells are extremely permeable to urea, others to glycerol: in subepidermal stem-cells of *Majanthemum bifolium*, water permeates 200 more rapidly than does urea which in turn, permeates 500 times faster than does cane sugar.

The second point, i.e. the varying degree of permeability under

different conditions, already became evident in the investigations of FITTING and others, referred to earlier, by their study of permeability in different seasons: in winter permeability is much less than in summer.

After stimulation we observe that some cells show even greater variations, This point will be dealt with in greater detail in connection with the physiology of stimulation, though here it should be mentioned that in different objects there are cells with protoplasm, the permeability of which becomes greatly enhanced after stimulation by different means, e.g. contact, electricity, etc. In the irritable stamens of *Berberis* it appears as though semi-permeability has made way for complete permeability. Not only does water exude from the cells, but also a number of substances in solution — according to some investigators even tannins. Yet it remains uncertain whether there is exosmosis of all the substances contained in the cell, and in any case there is this great difference as compared with dead cells that in the irritated stamens the phenomenon is reversible; after a short while semi-permeability is completely recovered.

During that time, the so-called period of relaxation, the cell is no longer irritable. A point of interest with regard to our discussion is that this recovery is a vital phenomenon which can occur only in the presence of oxygen and is thus obviously connected with respiration.

This raises another point, viz. that the penetration of substances into the living cell not only involves diffusion, i.e. a movement of molecules from a higher towards a lower concentration — as was thought in the days of DE VRIES and PFEFFER — but also a transmission in a direction opposite to that of the concentration gradient.

That absorption of water by cells, besides being a purely osmotic process, may be also a process caused by activity on the part of the protoplast and thus be dependent upon respiration and the presence of oxygen, was observed by Miss REINDERS [534] who worked on potato tissue in the laboratory of W. H. ARISZ [13] at Groningen. Later, when discussing the translocation of water, we shall find that intake of water by the roots may also be dependent upon the presence of oxygen.

Absorption of ions can also take place in a direction opposite to that of the fall of concentration. This fact was proved by tests with large unicellular algae, such as *Valonia*, in which it was possible to demonstrate that in the large vacuole, for instance, a more concentrated solution of potassium bromide was present than in the environment. In this case the result is that towards the inside of a semi-permeable boundary layer there is a higher concentration than in the environment and thus an osmotic potential is built up; in other words, a system has arisen which has a higher osmotic energy content than its environment. [1]) This is possible only at the expense of energy. The source of this must be the respiration of the living cell, since experiments have

[1]) At least if we may assume that there is no exchange between the chlorine and bromine ions.

shown that this process can occur only in the presence of oxygen.

The investigations carried out by F. C. STEWARD [424] and by D. R. HOAGLAND [241] in 1936 were the first to show that in fragments of potato tissue the absorption of salt is dependent upon respiration, or, in the words of the authors: "salt absorption seems to vary with the general level of respiration." Shortly afterwards it was demonstrated by HOAGLAND that this is also the case with the penetration of salts into roots; this will be discussed in Chapter V.

We should once more emphasize the fact that our views regarding the properties and functions of the different organs of the protoplast have undergone a change. As a result of the work of various authors, HÖFLER [262], LEPESCHKIN [372] and WEEVERS [704], it has become increasingly clear in respect of both sugars of various kinds and ions, that the behaviour of the outer boundary layer of the protoplast, i.e. the ectoplast, differs greatly from that of the layer bordering upon the vacuole, i.e. the tonoplast. So the idea of HUGO DE VRIES comes true; whereas the ectoplast normally appears to be more or less permeable to ions and sugars, the tonoplast is highly impermeable. Translocation through the latter is not case of diffusion, but a process of active secretion, the necessary energy for which is obtained by means of respiration. In the process of intake of substancess by the cell as a whole, on the other hand, an important part is played by adsorption.

The point of view set forth here explains, on the one hand, HÖFLER's test in regard to the so-called „Kappenplasmolyse", in which the cytoplasm swells as a result of too rapid penetration by certain ions, and on the other hand WEEVERS's observations concerning the rapid penetration of sucrose into the yeast cell. Formation of starch in sucrose solutions in the above mentioned experiments of BÖHM [55] and others can also be explained in this way. The same explanation holds good for the osmotic adaptation of *Nitella translucens* studied by L. S. WILDERVANCK [729].

W. H. ARISZ [13] and his pupils have followed the trend of this research. in various investigations. ARISZ makes a clear distinction between permeability s.str. where only plasmalemma and cytoplasm are permeated and transmeability where also the tonoplast is passed. It is particularly the latter process which requires energy.

Chapter IV

THE MOVEMENT OF WATER

(A) Introduction.

This chapter of De Vries's Textbook begins with a discussion of the fact that there are processes which require water, viz. growth, formation of carbo-hydrates and transpiration, of which the last-named plays by far the most important part. From the fact that in the higher plants there are certain organs whose particular function it is to regulate transpiration, i.e. the leaves with their stomata, Hugo de Vries[687] wants to draw the conclusion that transpiration must have a special significance in regard to life. In this connection he refers to: (1) the cooling resulting from evaporation, and (2) the increased velocity of the translocation of inorganic salts. The latter point will be discussed later; in regard to the former, opinions vary: it is uncertain whether this cooling is, in fact, quite so useful or indispensable as it was believed to be by de Vries and his contemporaries.

In my opinion, the problem should be put differently. Transpiration and evaporation are processes indispensable for the living plant in view of:

(a) the abundance of water in the living protoplasm and the cell wall;
(b) the fact that the formation of carbo-hydrates through absorption of the carbon dioxide from the atmosphere presupposes the existence of channels permitting the entrance of this gas.

It is inevitable that the channels for the entrance of carbon dioxide should at the same time act as escapes for water vapour; a plant with cuticular layers so thickly covered with wax as to permit no evaporation through these and, moreover, not possessing any stomata permitting transpiration, would be incapable of taking in carbon dioxide and could not exist as an autotrophic plant.

The actual state of affairs found in the higher plants which live in the air is a compromise to ensure a sufficient absorption of CO_2 and a not excessive transpiration and evaporation of water-vapour. The manner in which this compromise is achieved in the lower plants, such as the land Algae which do not possess any stomata is entirely different from

that in which it is achieved in the higher plants which do possess these. I cannot go into further detail here; I should only point out that these lower plants can resist extreme desiccation whilst they remain in a state of rest, and that subsequent moistening will revive them again. In nearly all the higher plants such extreme desiccation is possible only during a certain period, especially in the seed state.

(B) Transpiration.

When reading in the Textbook what DE VRIES has to say about transpiration, we are struck first of all by his well-balanced, logical argumentation with its clearly drawn distinction between cuticular transpiration or evaporation and stomatal transpiration; we are impressed by the lucid manner in which he pictures the influences of external circumstances on these processes. We are even inclined to wonder whether in fact, there has been anything new since that time, though we are fully aware of the immense amount of work done in this regard during the past fifty years.

It is only on re-reading this chapter that one begins to notice the serious lack of well observed facts expressed in terms of degree and number, such as the exact significance of the size and number of stomata, the effect of wind on evaporation, the action of light on the stomatal opening. All mere detail, one may say; true, but all the same indispensable for a closer understanding of the various physico-chemical processes.

If we bear in mind that it was not until the year 1898 that H. TH. BROWN [80] and F. ESCOMBE [162] made an experimental study of diffusion through the stomata and thus laid the foundations for the searching investigations carried out by SIERP [605], NOACK [462], BACHMANN [25], SEYBOLD [601], BRUNO HUBER [282], ILJIN [285], LIVINGSTON [384], MAXIMOV [415], SCARTH [571], GREGORY [211] and PEARSE [499], to mention only a few. If we call to mind that it was not until 1911 that PERTZ [502] and FRANCIS DARWIN [133] constructed the still rather primitive porometer, which instrument was later improved upon by VAN SLOGTEREN [609] and adapted for recording by M. PINKHOF [509], we realize to what extent our knowledge of transpiration and evaporation has advanced. But the nature of this advance cannot very well be stated in a few words; perhaps it might be summed up by saying that attempts are being made more and more to find a mechano-physical explanation of the diffusion of gas through the complex system of intercellular spaces and the small apertures in the epidermis, termed stomata.

A few facts must be recorded: first of all the difference between the transpiration of a leaf and the evaporation of a sheet of moist filter-paper. According to DALTON'S [131] law, evaporation $V = k\,(F—f)\,\frac{760}{P}\,S$. In this formula F stands for the maximum pressure of vapour at the

temperature t,f represents the pressure of water-vapour in the paper, S the surface of the object and P the atmospheric pressure.

This evaporation was determined experimentally with the aid of atmometers, i.e. porous pots connected with a water tank. It was found that the shape and the vertical or non-vertical position were important in connection with convection currents; it was found also that wind augments, the so-called saturation deficit (F—f); all these factors which rendered an exact formulation an extremely difficult problem. H. T. BROWN [80] and F. ESCOMBE [163] drew up the following formula in view of data obtained in tests with artificial and natural stomata $V = \frac{k.p.\pi r^2.n}{1 + \frac{1}{8}\pi r}$, in which V represents the quantity emitted per unit of time, p the deficit, r the radius of the aperture, n the number of apertures, 1 the length of the pore-tube, i.e., the depth of the stomatal pore in the leaf. But this formula, again, does not take into account all the various factors by which evaporation and transpiration are influenced. According to STEFAN'S [623] law, diffusion through round apertures is proportional to their surface if they are relatively large, but proportional to their circumference if they are small. This becomes understandable, to some extent, when one bears in mind the influence of the rim of the aperture where the diffusion-lines bend outward. If in BROWN and ESCOMBE'S formula the depth of the pore (1) is neglected, the formula becomes $V = 8k.p.r.n$; in other words, there is also proportionality to the radius and, consequently, to the circumference of the aperture. When, however, the apertures are small and are situated so closely together that one interferes with the action of another, the formula does not hold good; moreover, the stomatal aperture is not round but elliptic and their structure is very complex. In short, all these factors make the problem an extremely difficult and intricate one. I will cite only one instance; in *Betula*, a plant with relatively large stomata, M. G. STÅLFELT [621] found a transpiration amounting at the most to 70% of the evaporation of models of moist filter-paper of the same shape and size.

How do the stomata act? HUGO DE VRIES said the following: "the stomata are by no means equally widely open in all and any circumstances; on the contrary, the surrounding cells possess the property of now widening, now narrowing the aperture; this occurs as a result of the influence of light", and a little further on: "regarding the stomata the light plays the part of stimulant".

Thus, the fact that the opening of the stomata is the result of a rise in turgor of the guard-cells is not clearly stated here, nor is the action of the light explained.

In those days opinions on the mechanism of the stomata and the part which light plays in this connection were divided. As early as the year 1856, HUGO VON MOHL [435] advanced the theory that the chlorophyll content in the guard-cell indicated that by photosynthesis sugars were formed in these cells, which enhanced the osmotic value of the vacuole;

this led to the attraction of water and to increased tension in the wall, as a result of which the shape altered.

In the year 1881 these views were endorsed by S. SCHWENDENER [596], and the opposition by H. LEITGEB [370] met with no support. LEITGEB argued that when illuminated the stomata would open even in air that was free from carbon dioxide, so that absorption of this gas had nothing to do with it. He also drew attention to the importance of the accessory-cells, i.e. the cells situated next to the guard-cells. LEITGEB in studying these accessory-cells had found that when they lost their turgescence, the stomata would open even in the dark, from which he concluded that the guard-cells acted passively. DE VRIES, therefore, on the whole concurred with LEITGEB's views and spoke of a process of stimulation by light without defining this any further.

Later on K. LINSBAUER [382] succeeded in confirming the accuracy of LEITGEB's argument, but it was particularly the investigation by A. KÜMMLER [354] in 1914 which seemed to overthrow HUGO VON MOHL's theory altogether. KÜMMLER found that there are certain species of plants of which the variegated leaves contrary to the large majority of variegated varieties, possess guard-cells without any green plastids. In these varieties with chlorophyll-less guard-cells the stomata, nevertheless, open in the light, and this fact led KÜMMLER to the conclusion that it was not merely a case of an increase in osmotic value resulting from photosynthesis, but that some stimulating action of light was responsible for the increase in the osmotic value of the guard-cells. Obviously the accessory-cells were ignored by KÜMMLER in these observations.

It had long been known that guard-cells invariably contain starch grains; the few species where this is not the case, such as *Allium*, have a polyfructose, sinistrin, by way of carbohydrate reserve. F. LLOYD [385] and, later, W. S. ILJIN [285] found that the number of starch grains varied, i.e. it was small in the guard-cells of open stomata and larger in those of closed stomata. It also proved possible to determine the osmotic values of guard-cells and accessory-cells; in *Rumex Patientia* it was found by the American physiologists J. D. SAYRE [570] and R.C. WIGGANS [728] to amount at 13—14 atmospheres at night time in the guard-cells of closed stomata and to amount at 23—24 atmospheres in guard-cells of open stomata. The pressure in the accessory-cells was between 14 and 15 atmospheres. This makes it seem likely that the starch granules, which vanish as the stomata open, are converted into sugars possessing osmotic action.

In *Galium Mollugo*, S. STRUGGER [637] and F. WEBER [703] observed an obvious antagonism between guard-cells and accessory-cells; when the stomata were closed, the guard-cells contained starch and the accessory-cells did not, but when the stomate were open, the reverse was the case. So once again there was evidence of co-operation of the accessory-cells as had been assumed by LEITGEB.

It is found, moreover, that during the opening and closing of the

stomata changes in viscosity may be observed; the shape of the nucleus changes and while in closed stomata convex plasmolysis occurs, there is cramp-plasmolysis in open stomata; in other words, the p_H of the protoplasm has also undergone a change. Hence is is possible that the change in acidity is the primary result of the stimulating action of the light whereas the enzymatic processes are secondary.

Though we must not fail to mention that the results obtained by KÜMMLER were regarded as incorrect by K. W. PAETZ [493]. He found that the so-called chlorophyll-free stomata which he examined did contain minute quantities of chlorophyll in their plastids, as he was able to demonstrate by means of a fluorescence microscope. According to him, stomata which were truly non-chlorophyllous did not react to light, and the opening of the stomata was brought about by just those rays which are most strongly absorbed by chlorophyll. Since PAETZ is of the opinion that there is a great difference between the opening of the stomata in air containing carbon dioxide and the same process in air containing nothing of this compound, he all but reverts to HUGO VON MOHL's standpoint. In my opinion it would be curious if such slight traces of chlorophyll did yield enough photosynthates; moreover, H. SIERP [605] came to the conclusion that the shorter wave-lenghts of light have more effect on the opening of the stomata than longer wavelengths, i.e. the end of the spectrum where photosynthesis is strongest. This would seem to indicate that in transpiration the photo-chemical process is quite different from that in photosynthesis. Be that as it may, it is obvious that the analysis of the process which DE VRIES simply called stimulation by light has progressed a good deal, even though it is not yet fully understood.

Now we must consider another aspect of the problem of the opening and closing of the stomata. We saw that a rise in turgor causes the stomata to open whilst a fall in turgor causes them to close; the obvious question is: how does a change in the water content of the leaf affect the process?

In trying to ascertain the behaviour of the stomata of plants in a state of wilting, H. MOLISCH [436] and, later, H. BURGERSTEIN [95] obtained very dissimilar results with different species of plants. In some, the stomata closed during wilting, in others they did not, or only partial closure set in. C. G. P. LAIDLAW [360] and R. C. KNIGHT [334] experimented rather more carefully, making use of recording porometers; on cutting the stem of *Eupatorium* spec., for instance, they found that after a certain interval the stomata would open and subsequently close again. Closure is obviously the result of lack of water, but in regard to the opening of the stomata opinions are divided. Some investigators regard this as the result of the decreased tension in the accessory-cells, i.e. the cells through which the guard-cells appear to receive their water supply; others think of the pull exerted in the wood-vessels by cohesion forces which will be discussed later. In consequence of the cutting of

the stem, the continuous water column is broken, hence absorption in the part above the cut surface can occur more rapidly, the column of water having become much shorter.

Be this as it may, in any case it becomes obvious that the so-called water balance of the leaf, particularly of the cells surrounding the stomata, affects the width of the stomatal pore. The term "water balance" is of recent date and is used to indicate that in normal circumstances the amount of water absorbed from the soil and that given off by transpiration must to a certain extent be at equilibrium. When transpiration is greater that the supply, we speak of a negative water balance, which may presently lead to wilting and ultimately to complete desiccation, though in the early stages it is reversible.

That the surrounding tissue does in fact affect transpiration is clearly shown by the phenomenon that the stomata situated above the green parts of a variegated leaf open more widely than those situated above a yellowish-white part of the same leaf, despite the fact that in both cases they usually contain chlorophyll. This is probably the reason why the green parts transpire much more strongly than the variegated parts, a phenomenon which French authors, e.g. PH. VAN TIEGHEM [653] have termed "chlorovaporisation".

Investigations by the Swedish physiologist M. G. STÅLFELT [621] who made a careful analysis of the phenomenon of stomatal transpiration, show that the water balance of the leaves may change rapidly. He picked some leaves off a birch tree, placing them in a dark space which was saturated with water-vapour, and after an interval he ascertained the width of the stomatal apertures with the aid of a microscope. The stomata were found to be closed, but when the leaves were subsequently transferred to another dark space NOT saturated with water-vapour, they opened and closed again; this agrees with observations made by LAIDLAW and KNIGHT. STÅLFELT regards this opening of the stomata as a consequence of decreased turgescence on the part of the accessory -cells, through which the pressure on the guard-cells becomes less so that the latter open passively, phenomenon which he terms hydropassive opening. Subsequently, with what STÅLFELT terms a sub-optimal supply of water, a so-called hydroactive closing sets in, which generally begins at a loss of water of between 3 and 5% and which he regards as the result of a decreased turgor-pressure in the guard-cells.

In addition to these hydropassive and hydroactive movements, there are, in normal circumstances, also photoactive movements resulting from the illumination phenomena discussed above. Thus, according to STÅLFELT, in the morning there is first a photoactive opening movement which is reinforced by the hydropassive one when the leaf attains a less turgescent state; hence at approximately 10 a.m. the size of the pore is at its maximum. Subsequently the water supply becomes increasingly sub-optimal, causing the hydroactive closing movement to set in, which counteracts the photoactive movement. Thus a process of

regulation ensues, for each narrowing of the aperture by virtue of hydroactive closure results in a decrease of transpiration, which affects the water balance in a favourable sense; hence the width of the stomata is regulated more or less automatically. Is this regulation of the size of the stomatal pores the determining factor in regard to transpiration? In view of the above one would be inclined to answer this question in the affirmative forthwith, but we shall find there is still a good deal of disagreement in this score.

More than 50 yesrs ago HUGO DE VRIES referred to the daily march of transpiration which was supposed to be brought about by the co-operation of a number of factors; we shall see that since the analysis of this phenomenon has made considerable progress.

I cannot enter into a discussion of the innumerable investigations concerning the influence of external circumstances on transpiration: that would lead us too far into the more specially ecological field, though mention must be made of the principle of „limiting factors", this being a point of fundamental importance in transpiration.

About 100 years ago JUSTUS VON LIEBIG [376] discovered that when small doses of artificial fertilizer were administered, the yield of the crop would increase proportionally to the amount supplied, but as this is being stepped up, a point is reached where the increased dose no longer results in an increase in yield. In this connection VON LIEBIG referred to a factor as being in the minimum; at first the applied fertilizer was in the minimum, later another factor.

It was not until the year 1905 that a further elaboration of this idea of limiting factors was given by F. F. BLACKMAN [52] in consequence of an investigation by him and Miss MATTHAEI [414] concerning the process of photosynthesis. He made it clear that in a physiological chain-process of which one link must necessarily be followed by a certain other link, the velocity of the whole is determined by the velocity of the slowest link. This fact can best be visualized by thinking of the preparation of some product or other on a conveying belt.

Leaving aside the question for the moment, whether this principle should be regarded as absolute, i.e. whether in actual fact there is always only one limiting factor or link, it is at any rate certain that this principle has deepened our insight into a number of physiological processes. In regard to transpiration we have also begun to wonder what may be the limiting factor; in view of the above one might be inclined to regard the condition of the stomatal pores as such, though whether or not this is correct is still a moot point. A decision in this matter is hard to arrive at. For the transpiration of a plant is dependent upon the velocity with which water is taken up from the soil, secondly upon the velocity with which this water is moving through the plant and, finally upon the rate at which it is given off in some form or other. Disregarding the first two factors the time being, we must not lose sight of the fact that the evaporating surface proper consists of the adjacent surfaces of the

parenchymatous cells that are filled with living protoplasm and border on the intercellular spaces. Either this evaporation or the diffusion through the intercellular spaces to the stomata might constitute the limiting factor, as might also the passage through the stoma itself.

As early as 1908 objections were raised by F. E. LLOYD [385] to the view that the stomatal movements were of primary importance in the regulation of transpiration. In the case of certain desert plants he was able to demonstrate that the curve representing the intensity of transpiration by no means coincided with that of the stomatal movement. American investigators, e.g. B. E. LIVINGSTON [384] and others as well as the Russian physiologists ILJIN [285] and MAXIMOV [415] pointed out the importance of evaporation in the intercellular spaces. The term "incipient drying", which is much used in American literature on the subject, chiefly refers to this part of the process of evaporation. By concentration of the solution the osmotic value will rise and the vapour pressure will drop with all the consequences thereof.

Against the extreme view of LLOYD, who regarded incipient drying as the limiting factor and who underrated the importance of the stomata, objections were justly raised by O. RENNER [537] and others, and although American botanists on the whole defended LLOYD's views, the importance of the stomata was clearly proved by J. V. LOFTFIELD [389] and later in my laboratory by K. HARTSUIJKER [241]. Speaking generally, I am of the opinion that it is the condition of the stomatal pore which should usually be regarded as the limiting factor; experiments with the aid of porometers, coupled with measurement of transpiration, tend to show this most clearly, though there are cases in which the significance of the other factors should not be underestimated.

(C) Xerophytes end Halophytes.

A subject which has been repeatedly and extensively studied during the past 50 years is the transpiration and the whole physiological condition of various ecological types of which I will specially mention the xerophytes, or drought resisting plants, on the one hand, and the halophytes, or saltplants on the other. Here again, only a very brief discussion of the countless investigations into the question is possible.

The question as to what is really meant by a xerophyte or what pecularities characterize plants growing in dry soils and climates. is the subject of a great deal of controversy in which the problem of transpiration comes much to the fore.

Considering what is nowadays expressed by the term "water balance" i. e. the equilibrium between uptake and loss of water, we might summarize the result of this controversy by the Latin adage: "variis modis bene fit," in other words, one aim may be achieved in various ways, in this case the maintenance of a water balance to suit the particular kind of plant.

In this connection a part may be played either by excessive water absorption with the aid of very long, deeply penetrating roots, generally possessing strong suction pressure, or by excessive limitation of the water-loss by closure of the stomata, coupled with a reduction of cuticular evaporation resulting from an abundant covering of wax or a radical limitation of the surface (globular form of many Cactaceae). In the last named cases we may also find succulency, i.e. the presence of a tissue which retains much water and gives the plant the fleshy appearance of many so-called xeromorphous plants.

From experiments carried out by D. MAC. DOUGAL [397] it appears that an *Echinocactus* which was unable to absorb any water for 6 years, lost one third of its weight; SHANTZ's [602] expression, "drought resisting" describes the succulent plants most aptly.

This succulence is usually accompanied by a low osmotic value of the cell-sap, hence succulent plants are well adapted for existence in a climate where every now and then—albeit with long intervals—rain occurs or where the surface of the soil is moistened by dew and the plant is able to absorb this water with its superficial roots. Existence in a country with a dry, salty soil, on the contrary, is practically impossible for these succulent plants. There one will find xerophytes of a quite different type, whose exterior may show nothing out of the ordinary, but which have roots that go down especially deeply and whose tissues possess specially strong suction pressure.

As an example of the type with deep-growing roots we may mention *Alhagi camelorum*, a shrub with small leaves and thorns, which belongs to the Papilionaceae and which in the desert-like steppes of Asia minor is capable of reaching the water in the soil with its roots even at a depth of 25 meters. In other cases the plant may have particularly strong suction pressure in its tissue; *Peganum Harmala*, one of the Zygophyllaceae, has osmotic values of over 100 atmospheres; in the driest periods the plant remains in a state of permanent wilting.

Different again is the course of events in plants from the "macchia" in the Mediterranean regions; these plants transpire strongly while the stomata are open, but after closure of the stomata transpiration becomes greatly reduced as a result of the peculiar structure of the cuticle; these plants can resist a fair degree of desiccation.

ILJIN [285] and others have shown in regard to transpiration that the reaction of such xerophytes to a dry atmosphere is entirely different from that of the mesophytes which are not adapted for such extremes. In a dry period the stomata of the xerophyte will remain open much longer, so that carbon dioxide absorption can continue longer, which is undeniably an advantage to the species in the struggle for existence.

MAXIMOV [415] regards the ability to resist intensive loss of water as the most specific property of the drought resisting plants. The question which property of the protoplasm determines this resistance will be considered later in the discussion of resistance to low temperatures.

Here I will only mention the fact that small-celled tissue is an important factor in connection with this resistance: this greatly reduces the risk of mechanical damage to the protoplasm during desiccation and shrinking of the cells. Typical examples of such resistance to desiccation are found in various South-African species which were studied by D. THODAY [650]. *Myrothamnus flabelliformis*, one of the Saxifragaceae, can be preserved dry for a whole year and, will revive in one night after moistening. As a matter of fact, this is a fairly common phenomenon in the lower plants, such as the mosses and lichens.

Disregarding the earlier teleological views concerning the so-called xeromorphous structure of plants from dry regions, H. WALTER [697] tried to find a causal explanation for this kind of structure. He argued that in plants with a low water balance, the protoplasm will be relatively dry and this particular of the protoplasm will leadto a different structure of the cell-walls formed. If a mesophyte, i.e. a plant adapted for moderate loss of water, is grown under conditions of limited absorption, the result will be a specimen with more or less xeromorphous properties. Such modifications are at present generally considered to be non-heriditary, WALTER, however, assumes that, in the long run, they might become hereditary, but he does not produce any evidence to prove this assumption. In my opinion it is more likely to be a case of minor mutations with xerophytic properties adapted for a dry environment, but then, again, it is difficult to see how there could be any question of causality.

In his observations concerning the water balance, WALTER introduces the idea of hydrature. On analogy with the idea of temperature, he understands by hydrature the condition of a plant as regard its water-content; the hydrature is highest at the point of saturation and falls during desiccation. The hydrature of the protoplasm is at equilibrium with that of the cell-sap, of which the osmotic value is the indicator; the higher the osmotic value, the lower the hydrature. According to WALTER, drought resistance is based on the property of being able to resist a high osmotic value, i.e. a low minimum of hydrature, or the ability to maintain a certain osmotic value over a longer period. In the case of succulent plants the osmotic value is fairly low.

VON FABER [168] stated the interesting fact that species found on volcanic soils of Java and containing much alum, the so-called alum-plants, have a xeromorphic habit.

In ecological observations on the subject of xerophytes and halophytes, the theory of A. F. W. SCHIMPER [575] has played an important part. He elaborated this in his book "Pflanzengeographie auf physiologischer Grundlage", which was published in the year 1898. In this book mention was made for the first time of a physiologically dry soil, i.e. an environment which does contain water though its absorption is difficult for the plant because in one way or another the water is mechanically or osmotically bound. SCHIMPER regarded sea water, or

a soil saturated with sea water, as an environment of this kind and he came to the conclusion that the succulent aspect of plant communities of a brackish soil, the so-called halophytes, may be compared with the succulency of true xerophytes such as the Cactaceae. According to him, the transpiration of such halophytes was but slight, hence water absorption was limited and the accumulation of salts, which accompanies the absorption of water, was avoided.

On some points this reasoning is not altogether correct; even apart from the fact that water and salts are by no means always absorbed proportionally, it is obvious that in the long run a high concentration of salts is inevitable. But it is one of the characteristic pecularities of most halophytes that such salt accumulation can occur with impunity. In the leaves of *Avicennia*, a mangrove plant of the swampy tropical coasts, F. C. FABER [168] found an osmotic value of 160 atmospheres, at least during low tide.

A few years later SCHIMPER expressed a more logical opinion in his "Indomalayische Strandflora", when he stated that the typical halophyte was capable of accumulating a great deal of salts in its tissue and thus obtained sufficient suction pressure to absorb the necessary moisture from the so-called physiologically dry environment, i.e. sea water. In this connection it is rather interesting to mention VON FABER's observation that under certain circumstances mangrove halophytes transpire fairly strongly.

After VON FABER's work in the tropics, the transpiration of halophytes in the temperate zones has been studied repeatedly. The opinion voiced by VON FABER was endorsed by O. STOCKER [631], and when, as a result of the work of H. CHERMEZON [109] and of H. J. VAN LANGENDONCK [363], it became clear that the anatomical structure of the halophytes did not indicate xeromorphy, SCHIMPER's theory was generally rejected. Later, however, there were authors who wanted to see SCHIMPER's theory accepted again; research by J. BRAUN-BLANQUET [70] and his collaborators with respect to the halophytes of the Mediterranean coast showed that these do have a slight transpiration. In regard to the halophytes of the Frisian islands, E. SCHRATZ [586] came to the same conclusion, while M. J. ADRIANI [1] likewise supported BRAUN-BLANQUET in consequence of physiological experiments carried out by him in my laboratory.

The great difficulty lies in the question as to how the transpiration of different species is to be compared, neither the leaf surface nor the fresh weight constitutes a suitable measure. The investigations of ILJIN [285] and others, referred to earlier, showed that behaviour of plants varies considerably and that they overcome obstacles entailed by a dry atmosphere and little rainfall in different ways.

The seemingly succulent exterior of halophytes is by no means proof of their xerophytic character, but should be regarded as an instance of chemomorphosis, i.e. the result of the formative action of

some chemical substance. From investigations carried out in my laboratory by M. VAN EIJK [104] it appears that it is especially the presence of chlorine ions which causes succulency. Nevertheless, in his investigations concerning the water balance of halophytes of the North Sea coast and the Mediterranean region and after carefully weighing up the pros and cons, ADRIANI [1] arrives at the conclusion that the halophytes which he examined do possess the characteristics of xerophytes, at least if the term is interpreted in the sense of the most recent researches; in his opinion, SCHIMPER's theory still retains its value.

The idea of a physiologically dry environment can also be used with respect to moorland plants, giving an explanation of the fact that a number of such plants show a xeromorphous habit. The difficulty lies in the question as to why moorland soil should be regarded as physiologically dry. On the one hand this idea is based on the fact that in the early spring this soil has a low temperature which may hinder water intake; on the other hand FIRBAS [173] considers the intensive desiccation of the top layers of moorland peat in summer to be the chief factor.

(D) Water Absorption, Guttation and Bleeding.

The subject of transpiration is followed in the Textbook by a discussion of water absorption by the roots. At the time when the Textbook of DE VRIES was published it was generally believed that the root hairs played the major part in this process. Conclusive evidence, however, was lacking, despite a mass of circumstantial evidence, such as the absence of root hairs in roots which normally grow in water. After the year 1895 H. COUPIN [123] argued that the root cap was the special organ for the absorption of water, but the investigations carried out in 1936 by SIERP [605] and A. BREWIG [75] with the aid of modern methods provided definitive, experimental proof that water absorption is the function of the roothairs. Absorption of water in the region of the root hairs greatly exceeds absorption in the root top.

From investigations carried out with onions, *Allium Cepa*, HILDA F. ROSENE [552] also came to the conclusion that all root regions between the root cap and the bulb absorb water. In relatively young roots there is an unidirectional gradient of the distribution of water absorption velocity, the region of maximum absorption apparently is at the base. In older roots there are maxima in regions 40 to 60 m.m. from the apex. GREGORY [211] and WOODFORD [742] recently studied the NO_3-intake in the primary main root of *Vicia Faba* and found the intake to be greatest in the tip-zone and gradually diminishing as the base was approached.

It is understandable that in the year 1895 HUGO DE VRIES should stress the osmotic aspect of the problem of water absorption, since at that time hardly any thought was given to the important question as to how

the cortex of the root obtained the water absorbed by the root hairs. As we shall see later, the idea of suction pressure or suction tension which is used in modern research, was not wholly developed in 1895, although it was implied in the observations of DE VRIES and PFEFFER. RENNER [537] and later A. URSPRUNG [667] followed DE VRIES in going deeper into the idea of suction tension of the cell. In its simplest form the idea accounts to this, that the suction pressure or suction tension of the cell (S) may be regarded as equal to the osmotic value of the cell-sap (O) minus the wall-pressure or wall-tension (W). This becomes clear when we remember that, if an isolated cell is in a position to absorb water to an unlimited extent, it will continue do to so until the wall-tension balances the osmotic water-attraction of the cell content, when the suction pressure becomes zero. Conversely, the suction pressure will be maximal when the cell shows incipient plasmolysis, so that the wall tension equals zero.

The above suggested to URSPRUNG the idea that, if out of two adjacent cells one has greater suction power than the other, the former will draw water from the latter and this process will continue until their respective suction pressure has become equal. Assuming the suction pressure in the root to increase towards the centre, we would thus have an explanation of an inward movement of water.

Be this as it may, it is certain that in 1895 both water absorption by the root hairs and movement from these to the vessels were regarded as osmotic processes. In tracing the development of plant physiology since then we shall find that this conception was not altogether correct, or, rather, not altogether complete. This can be shown best by including the bleeding and guttation of plants in our discussion.

The phenomena of guttation and bleeding were well-known in 1895 It is expressly recorded by HUGO DE VRIES that nearly all species may bleed when placed in an environment saturated with water-vapour. Guttation is also referred to, and so are the conditions under which this phenomenon occurs: in this connection he mentions a warm, moist soil and an atmosphere rich in water-vapour. He draws attention, moreover, to the typical structure of the hydathodes and the epithem tissue situated beneath them. The structure and function of the former, the emissaria or hydathodes, was explained earlier by J. W. MOLL [437], professor at Groningen.

The fact that bleeding and guttation occur only in the presence of oxygen containing air is not mentioned. It is difficult to say who was the first to observe this fact, although it is expressly referred to by A. WIELER [725], who published his investigations in respect to bleeding in the year 1893.

The occurrence of diseases in the root when oxygen is lacking formed the subject of a monograph by J. W. M. VAN ROODENBURG [551] at a much later date.

In the year 1938 P. R. WHITE [721] made interesting experiments

with isolated roots in vitro of which he studied the root pressure.

HUGO DE VRIES does mention in his Textbook that both guttation and bleeding are the result of processes in the living root, and to this extent it may be said that he, too, regards the respiration of the roots as indispensable in this connection. DE VRIES refers to root-pressure, but does not express any opinion on the nature of this process. Nevertheless, at that time various theories regarding this subject had already been formulated, and many others have since been added. When in the year 1933 J. G. HEIJL [255] wrote a thesis on ,'the influence of external circumstances on the bleeding of plants", he mentioned no fewer than 9 different theories. To deal with all of these in extenso would lead us beyond the limits imposed here, though we must briefly refer to the views held by PFEFFER in this respect. These were published first in 1877, but did not become generally known until after publication of PFEFFER's "Handbuch der Pflanzenphysiologie" in 1897; they greatly influenced subsequent research.

PFEFFER's starting-point was the fundamental question whether it was possible for a living cell to absorb water on one side and excrete water on the other side. He mentioned three possibilities:

(1) unequal properties of the plasma membrane or plasmalemma;
(2) unequal osmotic values at two poles of the cell;
(3) similar conditions everywhere as far as plasma and plasma membrane are concerned, except for a difference between osmotically active constituents in the cell-wall.

In practice, the third scheme seems to be of little importance; schemata (1) and (2) have repeatedly formed the basis on which later investigators built up their theories. The 2nd scheme seems simplest, particularly when we make use of the equation for suction pressure, formulated by URSPRUNG [667]. Let us assume that at one pole of the cell the osmotic value (O) is 8 atmospheres while at the other pole it is only 2 atmospheres, the wall-tension (W) everywhere amounting to 5 atmospheres. In accordance with URSPRUNG's simplified equation S (suction pressure) = O—W, the suction pressure at one pole will thus amount to 3 atmospheres, at the other pole it will be —3 atmospheres; in other words at a pressure of 3 atmospheres water will be excreted there. But this cannot continue: at such a short distance the dissimilarity in osmotic values would soon be levelled off by diffusion; the movement of the water itself would cancel out the difference in concentration unless such differences were maintained at the expense of energy. Thus this scheme hardly amounts to an explanation: the maintenance of the difference in concentration, which the action necessitates, remains unexplained.

W. W. LEPESCHKIN [372] in the year 1906 made a study of the active water-excretion of *Pilobolus crystallinus* and the hydathodes of the Araceae, taking the 1st scheme, i.e. unequal permeability as his working hypothesis. But this while accounting for the excretion of a diluted

solution, did not explain the one sided absorption. Later, when discussing the bleeding of birch and sugar maple, *Acer saccharinum*, LEPESCHKIN assumed scheme (2) to be the correct explanation and regarded the breakdown of accumulated carbohydrates as the active power.

From various quarters weighty arguments were raised against the hypothesis of simultaneous absorption and excretion of water in one and the same cell; nevertheless, in the year 1926 URSPRUNG and G. BLUM [53] accepted PFEFFER's 2nd scheme as the explanation, when they believed they had observed what is termed "the endodermis jump". By this was meant the great difference in suctionpressure between endodermis and adjacent cells of the central cylinder. It was thought that the endodermis was the place to which water was drawn up out of the cortex and where it was pressed into the central cylinder which process was believed to cause bleeding and guttation. H. H. DIXON [139] and others, however, quite rightly pointed out that it is questionable whether the arguments of URSPRUNG and BLUM are based on actual facts. They wondered whether the difference in suction pressure at the two sides of the endodermis, which these authors observed, might possibly be due to the fact that in these investigations one was forced to work with thin sections of tissue, in which the original negative tension in the vessels had disappeared.

The school of PRIESTLEY [519] as well as W. R. G. ATKINS [20] hold an entirely different view; they regard the endodermis as an osmotic partition and all the tissue on the inside of it as one whole that should be viewed as one large syncytium on account of its protoplasmic connections. This would absorb water osmotically from the totally of cells situated on the outside of the endodermis, which, to all intents and purposes, is to be regarded as being saturated with water. It was assumed by PRIESTLEY that the contents of the vacuoles were disengaged during differentiation of the young xylem. These disengaged vacuoles as well as the dying protoplasm would be the only sources of mineral supply necessary in the process mentioned above. But experiments made by WIELER [725] as early as 1893 which showed the bleeding to occur in roots even when their tips with the adjacent 50 mm were cut away, had proved that PRIESTLEY's assumption could not be correct.

The views of D. SABININ [564] are in agreement with the assumption that the intake of water is a purely osmotic process. This Russian physiologist analysed the bleeding sap of decapitated plants that had been grown in water cultures and concluded that its fairly high concentration had connection with that of the water culture. But several cases are known to us, particularly among herbaceous plants, where this sap has a very low concentration and where the simple explanation of bleeding being an osmotic phenomenon does not hold good. It would have to be assumed that the concentration in the vessels of the central cylinder was high, but became diluted during translocation in apical direction when the dissolved substances were adsorbed by the wall.

In my opinion, the influence of the tension of oxygen, which has been observed by so many investigators, is an irrefutable argument agains the assumption of a purely osmotic process.

Before considering this last point more closely, it must be mentioned that in MÜNCH's [444] theory regarding the movement of sap in the sieve tubes which we shall deal with later, bleeding is also referred to. MÜNCH assumes that bleeding is the result of water excretion which takes place in the vessels during cambial growth. It is difficult to discuss his views at this moment, since the whole of MÜNCH's theory is involved; it will be better to do so when dealing with the translocation of organic substances in higher plants.

That in guttation and bleeding osmotic processes do play a part is shown by the fact that, by placing plants of watercultures with their roots in a concentrated solution, it is possible to bring both phenomena to a standstill and even to extract water from the plant. However, that the osmotic process is not the only one in guttation and bleeding is shown equally clearly by the effect of various external circumstances. Firstly by the effect of a rise in temperature, which already became apparent in the early investigations regarding the bleeding of the birch, published by J. SCHRÖDER [589] in 1869. When later this phenomenon was studied by J. G. HEIJL [255] and others with the aid of modern methods it became clear that it is by no means a mere effect of temperature on an osmotic process. A rise in temperature of 10° C has as little effect on an osmotic process as on the gas pressure and here the effect is much greater. In guttation and bleeding changes in temperature cause a change in rate according to VAN 'T HOFF's rule, i.e. a rise im temperature of 10° C almost doubles the intensity. Like other vital processes bleeding and guttation also have an optimum, a maximum and a minimum.

Secondly, the effect of the tension of oxygen points towards a vital process for under anaerobic conditions or in a state of narcosis bleeding and guttation will cease, hence the respiratory process is obviously indispensable. HEIJL made a curious observation, viz. that when at a certain temperature bleeding has been brought to a standstill by means of a narcotic, it will be resumed when the temperature rises. The effect of external circumstances might be made to fit in with PFEFFER's second scheme by the assumption that respiration is necessary for the maintenance of the difference in concentration at the two poles of the endodermis cells, but this is no more than a gratuitous statement.

Other investigators point out that electro-endosmosis may possibly play a part in bleeding and guttation but this has not been investigated at all. The fact that strong currents can cause the bleeding to cease may be explained in a different way.

Summa summarum: bleeding and guttation are not exclusively, and not even in the first place, osmotic processes, they are also closely connected with the life of the root and dependent upon respiration; in other words they are — at least in part — active processes brought about

at the expense of energy obtained in respiration. Although we do not yet know at all how this takes place it may for the time being be best to speak of active secretion of water in the wood vessels by the living cells which surround them in the root.

In this as in many other cases new facts were brought to light by the study of plant physiology in the tropics. In the year 1908 H. MOLISCH [436] studied the bleeding of various tropical trees during his stay at „'s Lands Plantentuin" at Buitenzorg, Java. He found that the action of a wound-callus played an important part in this respect; the high pressure, from 5 to 7 atmospheres, which he observed, is local and does not set in immediately after inserting the manometer, but after some weeks have elapsed. This is very different from of our trees, such as birch and maple, in the spring, when the sap appears immediately after cutting and the bleeding pressure is much less, although it rises immediately to its normal height.

In palm trees the wound reaction is particularly strong and the quantity of bleeding sap is sometimes very large; the coconut palm, *Cocos nucifera*, for instance, will yield approximately 8 L per day for about a fortnight; in other species of palms these quantities are even larger.

During the past few years P. M. L. TAMMES [642] made a close study of the bleeding of the Aren palm, *Arenga saccharifera*. If in inflorescence the spadix is stimulated for a few weeks by repeated tapping, the sap will flow out when an incision is made. The daily yield will be from 3 to 4 L of a cane sugar solution of approximately 15%. Provided that the cut is renewed repeatedly, the flow may continue for a year. the sugar is the result of the conversion of starch in the pith and, according to TAMMES, translocation takes place, not along the xylem but along the phloem. Hence the process in this instance is not the same as that of the bleeding of trees, discussed above. It would appear to be a pathological phenomenon similar to the secretion of a cane sugar containing liquid by tulips in which "toppling" occurs. This term denotes the sudden sharp bending over of the flower stem of tulips, which occurs under special growing conditions. This disease was studied in the year 1930 by MARIANNE PINKHOF [508] in the flower-bulb laboratory at Lisse in Holland.

(E) Upward Movement of Water from the Roots to the Leaves.

If we wish to survey the publications on this subject and the various changes of opinion in regard to it during the past fifty years, we should first thoroughly acquaint ourselves with the position as it was in the year 1895, though for a clear understandng of the subject and its research we have to go back even farther.

After the 18th century, when STEPHEN HALES [225] discovered the path followed by the water elevated in the stem, opinions concerning the

cause of this upward movement remained extremely vague. In the year 1868, for instance, when C. A. J. A. OUDEMANS [484] wrote his textbook, which may be regarded as the forerunner of the one he wrote in collaboration with HUGO DE VRIES, it was still customary to refer to a "vis a tergo" and a "vis a fronte". Root pressure was regarded as the former i.e. a force pushing upwards from behind, while the suction pressure of the leaves was supposed to be a force working from the top or the front. In addition, capillarity, imbibition, osmosis and atmospheric-pressure were mentioned as attendant factors, while, lastly, recourse was had to living elements in the stem.

The critical mind of JULIUS SACHS [565] soon realised that this so-called explanation was no explanation at all. It simply would not do to attribute the phenomenon to root pressure, seeing that no demonstrable pressure was to be observed in trees in full leaf, nor to transpiration of the leaves, since he observed that the conducting vessels were filled partly with air and partly with water. For these so-called JAMIN's chains, consisting of alternate colums of air and water, are not easily displaced. According to SACHS, imbibition was the sole remaining explanation, but this implied that water movement took place in the walls of the vessels, which were imbibed with water and not took place in the lumen, as was generally accepted. This was the view to which I referred in the Introduction. He assumed that these walls possessed a particular structure, as a result of which they offered little resistance to the water movement.

Despite the great personal prestige of SACHS, these views found little acceptance among botanists; J. BOEHM [55] consistently opposed them, though frequently with rather unfortunate arguments; It was pointed out, furthermore, that according to POISEUILLE's law, resistance to the translocation of water increased in inverse ratio to the 4th power of the diameter of the tube; hence in this instance it would have to be enormous, as the water would have to flow in the spaces between the micellae of the wall.

HUGO DE VRIES found that woody plants will wilt much more quickly if they are cut off above water and subsequently placed in water than when the cutting is done beneath water. In the former case air enters into the vessels during the cutting and obstructs the translocation — surely an indication that water movement occurs in the lumen of the wood-vessels.

In the year 1882 F. E. V. ELFVING [155] filled the vessels of cut twigs with molten cocoa butter and found that when this cocoa butter had solidified and the lumen of the vessels was blocked, the upward movement of water came to a standstill. It was objected by SACHS's followers that the fat might have denatured the wall of the vessels, rendering it unsuited to the movement of water, but in regard to the experiments carried out by L. ERRERA [160] in 1886 this argument could hardly be raised. ERRERA caused a solution of gelatine to be drawn up in the ves-

sels and when this had solidified, water movement was likewise found to be obstructed. This was also the case when a twig was pinched so tightly that the walls of the vessels became pressed together; moreover, the upward movement of water inside the vessels was observed experimentally by J. VESQUE [678].

About the year 1890, the general opinion was that SACHS's imbition theory was untenable, although his pupil, A. HANSEN [230], still tried to defend it. Then the old theory of co-operation of living cells in the stem was revived. This was elaborated further by E. GODLEWSKI [201], who declared that the medullary ray cells acted as pumping — and pressure — stations, though he had to admit that there was no evidence of this in the structure of wood. It was found, moreover, that movement of water in the stem was also possible in the opposite direction; this discovery by no means strengthened the theory. Nevertheless, J. M. JANSE [295] still tried to defend it and to work it out in detail.

GODLEWSKI's theory was open to experimental attack. In the year 1891 E. STRASBURGER [636] made experiments with trees whose trunks were about 20 m high and also with woody twining plants, in which he caused poisonous solutions to be drawn up to the top. He found that a solution of eosin, subsequently administered was also drawn up, although it could be assumed that all living cells in the wood had been killed by the poison absorbed previously. Thus the theory of co-operation of the living cells was discarded, though it must be admitted that these tests did not prove that in a stem which had been killed, the quantity of water translocated was as great as in a living one. Hence, in the Textbook by HUGO DE VRIES the assumption is still put forward that, in addition to root pressure and suction by leaves, there is also co-operation of living cells in the stem.

In 1895, E. ASKENASY [17], and a little later H. H. DIXON [139] independently from each other propounded a new theory based on the cohesion of water. They argued that, while water is given off by the transpiring leaves and is being withdrawn from the vessels, it is due to cohesion that the water column in these vessels remains unbroken and water in the stem rises. Water columns in which no air is dissolved, do not break, not even when subjected to enormous pull; this can be proved experimentally. According to this theory, the energy required for the raising of the water in the vessels is derived from the energy of the sun which causes water to evaporate. More energy is needed for the evaporation of water from the living cells if these have to obtain the necessary water from vessels under tension.

DIXON cleverly connected the structure of wood, in particular that of the Coniferae, with the cohesion theory. From what has been said above it follows that the supply of water can occur only in vessels with continuous water-columns; if air happens to enter anywhere into the vessels, there is no longer any cohesion and they remain useless for water translocation until they are once more filled with water. The function of the

bordered pits in vessels and tracheids was explained by DIXON as follows: if air has become dissolved anywhere in the water and an air-bubble is formed while the water is being drawn up, that particular tracheid becomes useless for translocation. However, the positive gas pressure in this tracheid, coupled with the pull exerted on the surrounding tracheids, which still contain water, will press the torus of the bordered pits against the aperture in such a way that complete closure ensues. Though one tracheid has thus become useless, water movement in the others can continue. This explanation holds good for the Coniferae, which have only tracheids but does not apply to plants which have only vessels in the wood, such as *Ficus*, for instance.

Although DIXON's theory has its strong points, they are offset by weak ones. Thus, it is very difficult to prove the existence of continuous water columns in the vessels of the wood of trees, a fact which carries the more weight since it was shown by the investigations of SACHS that in functioning splintwood air must be present in addition to water.

Besides fervent supporters of the cohesion theory, such as O. RENNER[537], there were those who, like M. NORDHAUSEN [466] and A. URSPRUNG [667] [444] took a more intermediate view between the cohesion theory and the assumption of the co-operation of living cells. In this controversy an important part was played by manometer tests, the value of which was critically reviewed by E. REINDERS [535]. I cannot discuss all this in detail, but would merely add that in various herbaceous plants the presence of continuous water columns is irrefutably proved. When a vessel is cut. air will suddenly rush into it —an indication that in the vessels of plants with strong transpiration a lowered pressure is obtained, though it remains undecided whether the pressure amounts to, say 1/4 atmosphere or whether a real pull is exerted. The latter is an essential factor in the cohesion theory, as was explained again by DIXON in the year 1910 in a detailed publication by which he tried to refute various arguments raised against the theory. But as already stated, it is by no means a simple matter to prove that pull experimentally. More than 25 years later E. D. PRESTON [517] attempted to do so in the following manner. In a specimen of *Fraxinus american*, a tree of which the wood shows annual rings with alternating wide and narrow vessels, panels of bark were carefully removed up to very near the cambium; subsequently a plasticine cup was affixed to the trunk and filled with Indian ink, an incision was made, and the rapid penetration of the ink or some other coloured solution was recorded cinematographically. If the width and nature of the conduits, through which such penetration occurs, are known, it is possible to deduce from the rate of penetration which of the three possibilities mentioned below is the cause. It may be that:

(1) a strong pull is exerted on a coherent column of water in the vessels, owing to suction by transpiration or evaporation in the leaves; or
(2) weak negative pressure is obtained in the vessels, owing to the fact

that during suction the cell-walls are pressed inward and it is due only to the elasticity of the cell-wall that the liquid can flow in when the incision is made; or

(3) the vessels are only half filled with water, the other half containing a space with a pressure varying between 1/10th and 9/10th atmosphere.

According to PRESTON the result of the experiments did not point to possibility (1), but it indicated that either (2) or (3) might be the case, so that in regard to species of trees possessing wide vessels, e.g. *Fraxinus america*, he was inclined to reject DIXON's theory.

Apart from this yet another objection was raised against the cohesion theory. Assuming that the theory is correct and that a pull is, in fact, exerted on the vessels, it must be admitted, nevertheless, that every now and again some vessels will become useless as a result of penetration of air. Do these ever become filled again with water and, if so, how? In the case of herbaceous plants one might think of root-pressure in this connection, but in high trees this would mostly be inconsiderable or insufficient. The observations of MÜNCH [444], referred to above, would come in useful here, since they seemed to prove that the elements of the medullary rays and, perhaps, also those of the cambium, are capable of pressing water into the vessels in a manner which will be discussed later. For this reason supporters of GODLEWSKI's theory still regard the medullary ray cells as indispensable elements for water translocation.

During the past few years ,however, research regarding the movement of water has taken a different trend. As HUBER [282] puts it, an attempt has been made to study the problem in the way in which an engineer views a problem connected with the movement of liquids. The engineer has to know two things: the capacity and the rate of translocation. If the diameter of the conducting vessels and the quantity of water moved per unit of time are known, calculation of the velocity from these data is a simple matter; one may also try to determine the velocity experimentally. In SACHS's days a solution of lithium salt was used; this was caused to be drawn up and then a spectroscope was used to show how far the solution had risen in a given length of time. Neither this method, nor the earlier one in which coloured solutions were caused to be drawn up, led to accurate results; the fact that one had to work with cut branches was an additional difficulty. With the aid of the lithium, for instance, SACHS found velocities of but a few meters per hour. Much later, CH. COSTER [122] working with coloured liquids, found very great velocities (between 50 and 150 m per hour) in tropical lianas.

To these methods a new one was added by HUBER: he heated the trunk in one spot and with the aid of a thermo-couple measured how quickly the heated water was translocated. In some trees, e.g. the oak, the ash, *Fraxinus excelsior* and the acacia, *Robinia pseudo acacia*, he found velocities varying from 25 to 44 m per hour; in others, such as the

beech, *Fagus sylvatica* and horse chestnut, *Aesculus Hippocastamum*, the rate was much less, varying from 1 to 2 m, and in the Coniferae the rate was lower still, viz. 50 cm.

This may, of course, have some connection with the width of the vessels and in this respect POISEULLE's formula may be called to mind. Lianas have particularly wide vessels with few partitions, so that, when a piece is lopped off water will even flow out. The oak has rings of wide vessels, formed in the spring, which alternate with rings of narrow ones, formed in summer; this is also the case in other species of trees with rapid water movement. The species in which movement is slow, e.g. the beech, have only narrow vessels, whilst the Coniferae have no vessels at all, but tracheids.

Trees such as the oak thus possess a special path for rapid water movement in the ring of vessels dating from the past spring; those dating from previous years are blocked by tyloses and have become useless. This fits in with the observations of DIXON, who said that the wider the vessels, the greater the risk of air entering into them, as a result of which they become useless, according to the cohesion theory. According to HUBER, it seems that the new wide vessels begin to be formed early in the spring so as to be ready by the time when the oak, whose leaves come out late, unfolds its buds. Whether or not this holds good for all species with rings of wide vessels is still unknown, but it is a fact that the leaves of the ash, *Fraxinus excelsior* and those of *Robinia pseudoacacia*, do appear late in the spring. From this point of view, specialisation of rapid translocation in a few large vessels has the advantage of greater velocity, though at the risk of earlier blocking of the path of translocation.

A few words on the capacity of water movement. HUBER compared the amount of leaves and the quantity of water given off with the diameter of the branch bearing the leaves. The calculation is only a rough one, since the walls of the vessels are included in it, but more or less serves the purpose. It is found that in most trees the relative conductivity, i.e. the quantity evaporated per unit of stem diameter, becomes less as one gets higher into the tree; in the birch, however, this is not the case.

Up to now we have only discussed the movement of water in the wood vessels, but it is clear that the supply of water to the living parenchymatous cells also requires extrafascicular translocation. Formerly it was thought that this was an exclusively osmotic process, but the above mentioned investigations, by HÖFLER [262], HUBER and others proved that the boundary layer of the living protoplast offers considerable resistance to the passage of water. The penetration of ions meets with even greater resistance and, as stated before, is brought about by active co-operation on the part of the living cell, which also plays a part in the movement of water.

The fact that calcium salts are deposited in the cell-wall indicates

that a not inconsiderable translocation of ions takes place in the parenchymatous tissue. This led S. STRUGGER [637] to ascertain whether it could be proved experimentally that translocation occurred in the cell-wall itself.

With the aid of fluorescent substances he at last succeeded in making translocation in the cell-walls visible, though not until various difficulties had been overcome. It was essential that the fluorescent substances should lack the property of accumulating in the protoplasm, at least under the conditions of the living cell. With a neutral reaction, berberine sulphate meets this requirement, and the sodium compound of oxytripyrenesulphonic acid was likewise found to have the required properties. With suitable objects, e.g. *Helxine Soleirolii*, one of the Solanaceae, it will thus be found that extrafascicular water translocation occurs to a considerable extent, and with relatively great velocity, in the intermicellary spaces of the cell membranes of the parenchymatous tissue. By crystallizing the berberine out with aid of a solution of potassium rhodanate, this phenomenon is rendered more specific and perceptible.

Summarizing, it may thus be said that the old imbibition theory of SACHS, although no longer of value with respect to translocation by the wood vessels, still retains its significance in regard to translocation via the parenchyma. The strong resistance in the narrow intermicellary spaces, referred to above, does not seem to constitute as serious a difficulty as former investigators used to think.

Chapter V

MINERAL NUTRITION

(A) The essential Elements and their Function.

When the Textbook appeared, it was known that carbon, hydrogen and oxygen are constituents of the compounds building up the cell-wall and that, in addition to these elements, the protoplasm contains nitrogen and sulphur, sometimes phosphorus. Numerous analyses of plant ash showed that the following elements are always present, potassium, sodium, magnesium, calcium, iron, chlorine, sulphur, phosphorus, nitrogen and silicon. In certain plants, e.g. some of the marine Algae, iodine and bromine are found and in what are termed the zinc-plants the element zinc was encountered. Moreover, very small quantities of lithium, aluminum, copper, cobalt, nickel, boron, strontium and barium were found in the ash from time to time.

It was natural to wonder whether all these elements were essential for the growth of the plant. An attempt was made to solve this problem with the aid of the water-culture method, which by 1895 had been known for almost fifty years. As a result of such tests it was concluded that of the elements present in the ash only, nitrogen, sulphur, phosphorus, potassium, calcium, magnesium and iron were essential and that the plant perishes when one of these elements is not available in sufficient quantities. It was also known that a deficiency of iron makes the plant chlorotic, hence it was concluded that non-chlorophyllous plants, e.g. Fungi, were able to do without iron. There was also a widespread belief that Fungi could dispense with calcium whilst for a number of seaweeds sodium was assumed to be indispensable.

What are the present-day views in this respect? In the course of years it became increasingly evident that water cultures based on the above findings did not lead to satisfactory results, but it was a long time before the conclusion was drawn that there are still other essential elements. The conclusions already drawn were incorrect, because the tests had been made with salts which were not altogether pure, but containing other elements as impurities.

Raulin's [529] observation in the year 1869 and Javillier's (296) in

1907 that zinc has a particular influence on the formation of spores in *Aspergillus niger* remained a disconnected fact without any further consequences. It was not until the years 1914/1919 that new perspectives were opened by the experiments of P. MAZÉ [418]. This investigator showed in water-culture experiments with maize that zinc and manganese had a favourable influence on the development of this plant and that no constant development took place when boron was excluded as much as possible. MAZÉ's work escaped notice in those parts of the world where French is not spoken, but investigations by various workers, for instance K. WARINGTON [700] drew attention to these facts.

W. E. BRENCHLEY [74] and WARINGTON found that disease symptoms occurred in the tissues of plants which were grown as far as possible under boron-free conditions. Investigations carried out later by MARIE L. LÖHNIS [387] at Wageningen showed that certain abnormalities may also occur in the formation of the reproductive organs. Later was observed the effect of a boron deficiency on photosynthesis and respiration of water plants; the primary action might be the part played by boron in protein synthesis, though no convincing arguments were adduced in support of this view.

Experiments carried out under the guidance of JOHA. WESTERDIJK [714] by J. C. 's JACOB [291], GRETA MES [421] and W. C. VAN GENNEP [197] in the Laboratory of the Willie Commelin Scholten Institute at Baarn (Holland) also demonstrated that boron was an essential element.

Now that the ice had been broken, observations concerning other elements were made by various workers. E. RENNERFELT [538] stated that manganese was essential for the growth of *Aspergillus*, as had already been argued earlier by the present writer's countryman H. J. WATERMAN [702], a pupil of BEIJERINCK [48]. Later it was found that copper. molybdenum and selenium were also essential for certain plants and it is probable that yet other elements will be found to be necessary, although the requisite quantities may prove to be even smaller. N. A. CLARK [115] for instance studied the function of manganese in the growth of *Lemna minor*, whilst H. D. CHAPMAN [108] has been able to produce typical copper deficiency symptoms. STEPHENS [625] and OERTEL [469] accumulated evidence to show that molybdenum is an essential food-element.

In recent years the new technique of tissue cultures has also been used to study this subject. In France studies were published by P. NOBÉCOURT [464] and by R. J. GAUTHERET [195], but especially in America the number of publications was great. I mention only those of P. R. WHITE [721]. Generally speaking the result of the plant tissue cultures was the same as that of the water cultures mentioned above.

The quantities required of the minor elements per 1 litre of water culture amount to no more than a few γ, that is roughly about one-thousandth part of the ordinary elements. Hence they are referred to as micro-nutrients, trace or minor elements, also as oligopleronts. The importance of these elements in seed was brought out by an

investigation carried out in the writer's laboratory by Mrs. C. H. VAN HARREVELD-LAKO [237].

Generally speaking, the functions of the micro-nutrients are as yet unknown. A deficiency of the elements precludes some metabolic process or other and results in abnormalities or disease symptoms: a boron deficiency leads to "heart rot" in sugarbeets; a copper deficiency results in the "reclamation disease" of cereals; "marsh spot" is a disease attributable to a deficiency of manganese. The significance of molybdenum for the binding of nitrogen by *Azotobacter* was brought into prominence by BORTELS [62]; BURSTRÖM [99] stressed the function of manganese in nitrogen assimilation. In relation to boron MARIE LÖHNIS considered the action of a particular hormone with a regulatory effect on the development of plants. In general it may be said that the extremely small quantity required indicates some function in hormonal or enzymatic processes.

As a matter of fact, the part played in the metabolism of the plant by the various nutrient elements of which the essentiality has long been recognized, is by no means clear either.

Even before the end of the last century it was known that a deficiency of iron brings about chlorosis in the green plant, and iron was thought to be a constituent of chlorophyll. This assumption was proved to be incorrect, and it was then assumed that some form or other of this element was necessary for the synthesis of chlorophyll. This has not been strictly proved, though it is now known that iron is a constituent of the respiratory enzyme and of the cytochromes which play a part in the transfer of oxygen. It has thus become clear that iron is an essential element, also for the plants without chlorophyll, e.g. yeast, in which -as we saw above -cytochromes are of considerable importance, even indispensable.

Just as phosphorus in the form of phosphoric acid takes part in the formation of the dehydrogenases and in that of the lipoids which help to form the boundary layer of the protoplasm, and is therefore absolutely essential, so magnesium is a constituent of the chlorophyll molecule. Moreover, magnesium is a constituent of the proteins which serve as reserve substances in seeds, and it probably has yet other functions, as is evident from the fact that, when this element is lacking in the culture solution, *Aspergillus niger* completely fails to germinate.

Potassium ions are found chiefly in the cytoplasm, though they do not seem to be completely absent in the nucleus of the cell. My own micro-chemical investigations in regard to the localisation of this element clearly showed the contrast between nucleus and cytoplasm: the localisation agrees with the assumed significance of potassium ions for the maintenance of the water balance and the hydratation of the protoplasm. As we discussed above in Chapter III, potassium is also found to play a part in the formation of the boundary layer of the protoplast and in view of this it is understandable that without potassium no plant development is possible. An interesting fact is that, according

to DELEANO [135] potassium ions are transported to the roots of annual plants at the end of the vegetation period and excreted, quite in contrast with calcium and magnesium ions.

Experiments have shown that calcium is indispensable for the life of the higher plants. This probably is in some way connected with the function of calcium in the boundary layer of the protoplasm, where it acts as the antagonist of the potassium ion. The assumption that for some of the Algae calcium is not essential is at variance with this fact, and the possibility cannot be ruled out that quantities so minute as to escape observation may be present also in those instances. Calcium is mostly present in the form of oxalate or other salts of organic acids, e.g. malic acid; in the cell-wall it is mostly present as a compound of pectic acid, though in Fungi this is not the case. In this particular instance it has been assumed that calcium is not essential as a nutrient element.

It would lead us too far to deal with all this in extenso, but there is one element which appears to be non-essential in the majority of cases and which I would like to consider somewhat more closely, viz. chlorine.

Whereas for the growth of the majority of land plants chlorine is not regarded as essential, and professional growers, supported by the experimental research of H. DE JAGER [292] even speak of the harmful effect of sodium chloride on most of the cultivated plants, it was found to be always present in halophytes, though its essentiality for these plants was not strictly proved.

In Chapter IV we discussed the question whether or not halophytes have a decreased transpiration; as stated, opinions vary in this connection, but apart from this the fact remains that in most halophytes the succulent type is stronger as the proportion of the entire complex of salts present in sea-water increases in the soil. Among these salts sodium chloride takes chief place, but ions of magnesium, potassium, etc., are also present. The question arises, therefore, as to whether all those ions are equally responsible for the fleshy structure, or whether this is due to one ion in particular.

This question may be solved by means of experiments in which such halophytes are grown in water cultures to which, besides the absolutely essential elements, the salts referred to, and so their ions, have been added in varying quantities. During the first few decades of the present century B. KELLER [318] tried to ascertain the facts, and it was upon his results that H. WALTER [697] based his theory that a high osmotic value of the cell-sap has shrinking effect on the protoplasm, causing a more xeromorphic structure to arise. In his opinion this was offset in the halophytes by the swelling action ascribed to the ions of the sodium chloride solution.

The experimental research of M. VAN EIJK [166], carried out in the Amsterdam laboratory for plant physiology, later showed that this is only partly correct and that the swelling action is a peculiar property

of the chlorine ions. If the degree of succulency (S) is expressed by the equation $S = \frac{\text{total water content}}{\text{surface}}$, it will be found that when the marsh samphire, *Salicornia herbacea* is grown without additional salts, this value S is 33; when grown with additional sodium nitrate it is 34, but on addition of sodium chloride, potassium chloride or magnesium chloride respectively, it will be about 45. From this it follows that the succulency of *Salicornia herbacea* is a case of chemomorphosis, brought about by the chlorine ions.

(B) Absorption in the Tissue.

Next we come to the question as to how the various elements are absorbed in the tissues. In 1895 all that was known in this respect was that the plant could only take substances in solution. The question was then more or less ignored, and it was taken for granted that salts in solution were absorbed and taken in together with the water, as though the boundary layer of the living protoplasm were made of filter-paper. This is the more surprising as the phenomena of diffusion and osmosis were well known at the times. HUGO DE VRIES pointed out that the protoplasm of the root-cells has a regulatory action and allows some salts to permeate better than others. Specific pecularities play an important part in this respect; owing to these pecularities different species growing in the same water may show a totally different ash-composition, both quantitatively and qualitatively. That salt absorption is not always a function of the water taken up by the plant was proved later by MÜNSCHER's [445] experiments. By comparing oat plants grown in a dry greenhouse and in a moist environment it was found that the former absorbed double the quantity of water per gram of ash-constituents. BÖTTICHER [57] and BEHLING [37], however, stated a close correlation between transpiration and calcium-ion absorption.

Recent investigations by JENNY [298], OVERSTREET [488] and AYERS [23] made it probable that not only dissolved substances are used and that direct absorption of ions from a fixed condition by means of interchange is possible. Surface migration of ions and contact depletion of barley roots was revealed by radioactive indicators.

Returning to the question as to whether the ions or the undissociated salts are taken up, and taking into account the highly diluted solutions which are generally present in the neighbourhood of the roots and the high degree of dissociation of these solutions, it seems most likely that the ions as such are absorbed. This probability becomes a certainty in view of the investigations of E. PANTANELLI [495], GLADYS M. REDFERN [533] and W. STILES [630], which prove that cations and anions need not be taken in to an equal, providing that this unequal intake is accompanied by a liberation of ions by the plant, so that on the boundary surface no great differences in potential arise. In the case of an excess intake of

cations, the plant seems to release H-ions; in the event of an excess intake of anions, it appears that carbon dioxide is evolved. Hence it is clear that by virtue of this process the degree of acidity of the surrounding solution may undergo considerable modification, and such has been proved to be the case.

As to the intake of ions generally, it has already been pointed out that W. J. V. OSTERHOUT [482], MATHILDA M. BROOKS [77], and other investigators experimented with large unicellular Algae, e.g. *Valonia*, in which the concentration of various ions in the vacuole may be higher than that in the surrounding water. Hence, during intake into the vacuole such ions may have been moved against a concentration gradient, thus setting up an osmotic potential possessing energy. As we discussed above this phenomenon is possible only at the expense of energy and this is in keeping with the view expressed by HOAGLAND [261] and STEWARD [629] that the energy requirement has been met by respiration. HOAGLAND varied the respiration by means of a change in temperature or in oxygen tension and found that the intake of ions ran parallel with it.

In a sense H. LUNDEGÅRDH [393] and his collaborators set to work the other way round; they varied the intake of salts by using different concentrations and then determined both the quantity of ions taken up and the quantity of carbon dioxide evolved. Their conclusion was that in the roots there is a so-called basal or fundamental respiration which occurs in an environment where there is neither intake nor setting free of ions. At a given temperature this fundamental respiration is a minimum value, and the respiration rises above this minimum when ions are either taken up or given off. LUNDEGÅRDH further stresses the view that it is the anions only which are taken in and set free at the expense of energy, but this view is opposed by HOAGLAND and STEWARD. LUNDEGÅRDH made an attempt to determine the electric potential of the boundary of the root versus nutrient liquid. From the predominantly acid character of this interface he suggests that the boundary surface of the protoplasm of root epidermis or root hairs has to be considered. On theoretical grounds it may be inferred that the potential of this interface is set up by the pressure of the H-ions therein; in other words, a root in a water culture may be compared to a hydrogen electrode. DONNAN'S [144] equilibrium holds good only for uncharged membranes and is therefore, of no real significance in this particular instance; on the other hand the movement of ions through the membrane is here a directed one, and thus the laws formulated by the present writer's countryman, the physicist F. A. SCHREINEMAKERS [587], apply.

In slightly acidified water ($p_H = 5$), a potential of about 200 mV arises at the boundary surface, and thus it may be concluded that this surface is formed by acid substances such as phosphatids, which were also mentioned in connection with BUNGENBERG DE JONG'S [92] work. The view that the surface of the protoplasm has a truly membraneous character fits in with this conclusion. By measuring potentials in diluted

acids, LUNDEGÅRDH found that the plasma membrane of wheat roots may be charged with H-ions up to a maximum where the $p_H = \pm 3$. This self-charge seems to act as a protective wall around the living protoplasm and is thus of fundamental importance for normal metabolism. If the environment also has a p_H equalling 3, the equilibrium is broken and damage ensues, which was shown to occur by KAHLENBERG [307] and TRUE [659] in the last years of the 19th century. According to LUNDEGÅRDH, this tension between membrane and environment will cause cations to be strongly attracted, whereas anions can be taken up only at the expense of energie.

As already stated, LUNDEGÅRDH worked with the formula:

$$R_t = R_g + k.A.,$$

in which R_t represents the total respiration, R_g the basal respiration A is the quantity of anions taken up at a certain temperature, and k the factor which indicates how many molecules of carbon dioxide arise in the respiration required for the intake of one anion molecule. In tests on oat plants carried out by LUNDEGÅRDH and R. BURSTRÖM [99] the value of k was found to vary considerably: for the chlorine ion it lay between 2,8 and 4,0, for the nitrate ion between 1,5 and 3,5 and for the sulphate ion it was about 12. To ascertain whether similar values were to be found also in other controls, M. VAN EIJK [166] made experiments with *Aster Tripolium*, a halophyte. As already mentioned, these halophytes have a high intake of salts, particularly of sodium chloride, and this intake may be affected from an environment that is poor in oxygen, as is shown by the degree of oxidation of the iron compounds in such soils. VAN EIJK working in the writer's laboratory, found that the value for the k in the sea starwort *Aster Tripolium*, was much lower than for oat plants: for the chlorine ion k was 0,108—0,56, for the sulphate ion 0,32—1,0. In view of his results it would seem that the intake of ions in the halophytes requires less energy, which may be of ecological significance in soils which contain but little oxygen.

In this discussion on the intake of ions, the exchange of ions must not be forgotten. This was brought into prominence by the experiments with radio-active potassium carried out by F. C. BROYER [83] and R. OVERSTREET [488]. If barley seedlings are placed in a solution containing both ordinary and radio-active potassium ions, the latter after a time will also be found in the root. This is caused by an exchange for ordinary potassium ions, apart from the intake of radio-active ones. If roots of plants that are rich in potassium are placed in a solution containing potassium ions, their concentration in the solution does not diminish, so there is no apparent intake or any setting free of ions. But if roots with a strong potassium content are placed in a solution with radio-active potassium, this will, after a while be found in the tissues, so that ane xchange must have taken place. Similar tests were carried out by HEVESY [254], BIDDULPH [49] and collaborators with the radioactive isotope

of phosphorus. Recent research of this kind also shows that giving off of ions from the living roots takes place. LUTTKUS [394] and BÖTTICHER [57] point to the fact, already mentioned above, that an amount of potassium ions which had been absorbed is given off by the roots if the plant is subjected to different conditions. In my opinion the process of salt intake is a dynamic and not a static one.

MACHLIS [400] studied the effects of respiratory inhibitors, potassium cyanide, sodium azide, potassium iodoacetate and potassium malonate on respiration and on the intake of radio-active bromide using excised barley roots as objects. He also found the inhibiting effect of malonate and iodoacetate to be largely surmountable by the addition of certain organic acids. This result supports the postulate that an organic acid respiratory cycle is intimately connected with salt accumulation. Cytochrome oxidase is the oxygen captivating enzyme probably affected by the cyanide and azide while the iodoacetate and malonate block another part of the organic acid cycle. MACHLIS also showed a gradient in respiratory activity in excised barley roots, the respiratory rate decreasing with increasing distance from the root apex. Later we shall see that this agrees with results obtained in other experiments showing similar gradients in salt absorption. HOAGLAND [261] in his „Lectures on the inorganic nutrition of plants" concludes that there occurs apparently some preliminary combination of protoplasmic constituents with the solute and it is almost impossible to avoid some concept of ion exchange as part of the process of salt accumulation.

In connection with this subject we must not lose sight of the fact that the oxygen required for the additional respiration may have a function both in the intake of ions into the protoplasm and in their conversion for further metabolic processes, as also for their translocation from cell to cell.

That the additional respiration might play a part in this conversion is considered unlikely by LUNDEGÅRDH [393], since for the chlorine ions, which are not converted any further, k is greater than for the nitrate ions. which are. Nevertheless the observations of others do point to this possibility.

In relation to the deposition of ions in the cell-wall and the ectoplasm, adsorption plays the most important part. The adsorption isotherm is an exponential function with the equation $y = k.c^m$, in which y stands for the concentration of the substance adsorbed, c represents the concentration in the surrounding fluid while k and m are constants. This function states the fact that an intake from a diluted solution is relatively more intense than an intake from a concentrated one. But this absorption equation does not throw any light on the further translocation to the inner part of the root tissue. In his investigations concerning the intake of phosphates by the roots of sugar cane, T. H. VAN DEN HONERT [267] justly emphasizes the point that the adsorption isotherm reflects the equilibrium between the concentration and the quantity adsorbed,

whereas with the intake of salts the connection between the concentration of ions and the velocity of their translocation into the tissue should be considered. Apart from adsorptive binding ,there must also be some mechanism for inward translocation, which as VAN DEN HONERT suggests might take place on a conveying belt system from cell to cell. But we are in danger of losing sight of the chronological order of the investigations.

In 1895 little attention was paid to the way in which this translocation to the xylem elements takes place. Mention was made of diffusion, but the difficulties caused by the semipermeability of the protoplasm were forgotten or ignored. It was not until 1910 that the experimental research of J. H. RUFZ DE LEVISON [558] showed that for various ions the endodermis is the boundary beyond which they cannot penetrate farther into the root. In order to reach the central cylinder, ions have to pass the protoplasm of the endodermal cells, since the Casparian strip prevents any translocation along the cell-walls, which are perpendicular to the root surface. This led to the assumption that in the cortex of the root such translocation did take place along the cell-walls. Many years later the above mentioned investigations by STRUGGER [637] and ROUSCHAL [555]. who demonstrated that a translocation of ions occurred in the intermicellary spaces of the cell-walls, enhanced the likelihood of this assumption. All the same the possibility cannot be ruled out that once the ions have been taken up into the protoplasm, they may be translocated with the aid of protoplasmic streaming movements, according to the hypothesis of DE VRIES, and need not be exclusively dependent upon diffusion. In accordance with the conclusion in the final part of Chapter III this translocation may take place in the cytoplasm along the vacuole, so no transmeability is necessary. If MÜNCH's and PRIESTLEY's idea that, various cell tissues form a kind of symplast by virtue of protoplasmic connections, proved to be correct this manner of translocation would have even greater efficiency.

There is still the question as to how the parenchymatous cells of the central cylinder pass on the ions into the xylem vessels. This may occur by means of active secretion, as is the case with the secreting glands, but it may also be that the hypothesis of A. S. CRAFTS [124] and F. C. BROYER must be accepted. These authors base their views on the fact that the cells of the cortex are well provided with oxygen, owing to the great number of intercellular spaces, whereas the cells inside the endodermis are not so provided. Hence the cells of the cortex would be capable of an active intake of salts, whilst those of the central cylinder were not — an attractive hypothesis, but against whose applicability in this case different objections can be raised.

Whichever view may prevail in the end, the fact remains that the concentration of ions in the vessels may be higher than that in the environment of the roots, so that somewhere this osmotic potential must be produced at the expense of energy.

(C) Plants growing on calcareous or silica Soils; Occurrence of Species in Connection with the p_H of the Soil.

In view of what has been stated above, it seems desirable to cover in this chapter the connection which exists between vegetation and degree of acidity of the soil. Practical experience of the past 25 years has shown that this connection does exist, but we shall deal with the question on a somewhat higher level and discuss this particular aspect of ecology — or environmental science, as it is called nowadays — a little more fully. At the outset it must be stated that, whereas formerly attention was focused exclusively on the occurrence of different species, latterly plant associations or plant communities have been given greater prominence.

As early as 1836 the botanist F. UNGER [666] observed in the Tyrol that the limestone mountains have a flora which is wholly different from that of the slate-stone mountains in the immediate neighbourhood. He expressed this by saying that the species of the one flora were bound to limestone, those of the other to slate-stone. This expression on the one hand suggested possible effects due to the chemical nature of lime, while on the other hand the physical influence of slatestone might equally well exert a certain influence' Soon the question: is the cause of this difference in flora a chemical or a physical one, became the main point.

THURMAN [652], upon his investigations in the Jura mountains, declared himself in favour of the second alternative, which 50 years later, in 1901 was elaborated further by G. KRAUS [347] in respect of the limestone mountains of Central Germany. The chief argument was that a calcareous soil dries out more quickly and thus gets warmed sooner in the spring.

The opponents of the physical theory stressed the fact that a calcarous soil has a poisonous effect on the so-called calciphobes, a phenomenon which was studied by GRANDEAU [210] with respect to the sweet chestnut, *Castanea vesca* as early as 1874. VALLOT [669] proved that the calciphobeous *Pinus pinaster* can keep alive only if the soil contains less than 3 %calcium carbonate. The fact that the sweet chestnut does grow in a calcareous soil if grafted on to the oak indicates that it is the effect of the soil on the root, which is of particular importance in this instance.

As regards natural vegetation, another factor, to which attention was drawn long ago by the Swiss botanist C. W. NAEGELI [449] has to be borne in mind, viz. that in nature there is always competition in the struggle for existence, so that one species will attempt to oust another.

There were divergent opinions as to exactly how this chemical action referred to above should be pictured. FLICHE [179] and GRANDEAU observed that *Pinus Pinaster* becomes chlorotic when grown in calcareous soil. Its ash is rich in lime, though poor in potassium and iron, and this fact might provide an explanation of its poor growth in calcareous soil. It was later demonstrated by W. MEVIUS [423] that a potassium deficiency produces totally different disease symptoms, but with regard

to iron this was harder to prove, the more so as the chlorosis could be cured by spraying the parts above ground with a diluted solution of iron salts. Hence the iron deficiency is probably only a secondary phenomenon. ILJIN [285] stated that these chlorotic plants show a totally different metabolism viz. an abnormal production of citric acid and soluble nitrogen compounds.

Weighty arguments in the controversy were adduced by experiments with strongly calciphobous species of *Sphagnum*. In 1898, OEHLMANN [468] found that to these peatmosses calcium carbonate is much more harmful than a solution of calcium sulphate or nitrate; later MEVIUS succeeded in proving the same with regard to the likewise strongly calciphobous *Drosera* species. The reverse was also found to be true; truly calcicolous species do not thrive when the element calcium is present in the form of calcium sulphate. It is evident that no thought must be given to any specific action of the calcium ion. The Dane RAVN[530] was the first to express the view that calciphobes needed an acid soil, whilst calcicoles required a neutral or weakly alkaline soil. But RAVN's publication, written in Danish, remained unread in the rest of the world; hence this view was stated anew by H. MOLISCH [436]. The American botanist WHERRY [719] was the first to carry out conclusive experimental research in this field with various ferns. He showed that the p_H may show considerable variations in adjoining plots of ground. Important in ecological respect are OLSEN's [471] studies on the hydrogen ion concentration of the soil and its significance with regard to the natural distribution of plant communities. In Holland the dunes provide a good opportunity for study, for there are particularly great differences in the degree of acidity between the recent outer dunes and the older inner dunes. The former are rich in lime, owing to their high shell-content, the latter are poor in lime. Thus the p_H may vary between more than 7 and approximately 5 and in the peat soil in the dune valleys it may be even lower. The plant associations vary accordingly; for instance on soil with a high pH associations belonging to the Bromion are found, whilst on a more acid soil the Querceto-Betuletum or Corynephoretum are encountered. This point, dispersion of associations, which was studied by the present writer on the isle of Goeree, is now a matter of general interest to plant sociologists of our country.

I can only briefly refer to the question as to how this difference in the degree of acidity may arise; I have already pointed out the importance of a high shell-content of the soil or the presence of lime-stone in the sub-soil; but how do soils like that of peatmoor obtain their high degree of acidity? L. G. M. BAAS BECKING [35] points out that in the regions near the sea the rain brings a quantity of sodium chloride into the soil, which should not be underrated. The explanation of the degree of acidity, which in moorland soil varies between a p_H of 3 to 4, may be that in peatmoor soil the sodium ions are adsorbed on the cell-wall of the peatmoss and exchanged for H-ions. The presence of peat moors

in the neighbourhood of the sea might be explained in this way.

From a purely physiological viewpoint a more interesting question is why the degree of acidity is of such fundamental importance to the plant. In this connection it should, perhaps, be borne in mind how the permeability of the boundary layer of the protoplasm may be affected. MEVIUS [423] suggested that too high a p_H might be harmful, as the permeability might be enhanced to such an extent that there would be too great a penetration of ions which would lead to overwhelm the protoplasm.

But this does not provide an answer to the question as to why an environment with too low a p_H may be harmful, a fact which has repeatedly been proved by experiments. KAHLENBERG [307] and TRUE [659] pointed out that dissociated acids have a lethal effect at $\frac{N}{6400}$, i.e. with a p_H of approximately 3,8.

An explanation might be found in LUNDEGÅRDH's [393] views discussed above, viz. that the boundary layer of the living protoplasm is formed by self-charged phosphatids, by means of which a protective layer in relation to the environment is obtained. Such an electric tension between membrane and environment, however, can be maintained only as long as the p_H of the environment does not fall below a certain value or rise above another value, which values depend upon specific differences. Thus it is curious that the boundary layer in respect of the vacuole can resist a far higher degree of acidity, a p_H of 1 to 2. In the so-called calciphobes the boundary layer of the protoplasm in the root hairs resists a p_H of 3, 5—4, that of calcicoles, plants of alkaline soils, on the other hand resists a p_H of 7 to 9.

(D) Photosynthesis; Introduction and Discussion from a physiological Point of View.

Before reviewing the work done during the past 50 years in regard to this aspect of plant physiology, I will briefly summarize the knowledge attained in this respect about 1895.

Generally speaking, it may be said that the Textbook of DE VRIES more or less reflects what has been known on this subject since the work of JULIUS SACHS [565].

(1) Photosynthesis occurs exclusively in the green parts of the plant and more particularly in the chloroplasts;
(2) Photosynthesis takes place only in the living chloroplasts and at temperatures between the minimum and the maximum;
(3) Photosynthesis occurs exclusively in light and most intensely in the red region of the spectrum, there being also a weaker maximum in the blue region;
(4) During photosynthesis, carbon dioxide is taken in from the atmo-

sphere and oxygen is liberated into it, in approximately equal volumes;

(5) As appears from the increase in dry weight during photosynthesis, the process also entails binding of water in the formation of the photosynthetic product. This product is a carbohydrate, probably glucose in the early stage, later starch. This may be represented by the equation:

$$6CO_2 + 5H_2O = C_6H_{10}O_5 + 6O_2$$

Although HUGO DE VRIES does not mention it, A. VON BAEYER [26] as early as 1870 suggested that during photosynthesis first of all the simplest carbohydrate, formaldehyde HCOH, might be produced.

As regards pigments, it was known that, besides chlorophyll, carotene ($C_{40}H_{56}$) and xanthophyll ($C_{40}H_{56}O$) were invariably present, and as early as the year 1864 STOKES [632] mentioned two green chlorophyll pigments, for which he gave different elementary formulae.

So we see that the physiological process was known as far as the initial and final stages are concerned and that there was at least some knowledge of the physical conditions of light and temperature under which the process takes place. At the same time there was a complete lack of understanding of the real nature of the physico-chemical process.

In what respect do we possess greater knowledge and deeper understanding to-day? The subject has been considered so extensively and from so many different angles that a purely chronological discussion of it as a whole would only cause confusion. For that reason I shall devote a separate discussion to each of the various ways in which the problem has been tackled. This naturally leads me to group the body of research in question under a number of headings, viz. physiological, physical and chemical investigations, although I am fully aware that it is by no means alsays possible to draw a hard and fast line between them.

Physiological investigations are mainly confined to ascertaining the influence of external circumstances on the process of photosynthesis; they can be subdivided into investigations concerning the influence of temperature, light and chemical substances-particularly poisonous ones.

As regards temperature not much more was known in 1895 than that, with a rise in temperature, photosynthesis would increase slowly from the minimum at about 0° C (in Lichens it lies below that temperature) to the optimum at approximately 30° C and would then fall slowly to the maximum at about 40° C. Minimum and maximum were the so-called cardinal points.

The investigations carried out by F. E. BLACKMAN [52] and Miss MATTHAEI [414] in 1904 shed new light on the problem. Cut leaves were first brought to a certain temperature and subsequently the reduction of carbon dioxide in the light was determined by means of

KREUSZLER's [349] method, in which a current of air with a known content of carbon dioxide was introduced into the sealed space containing the leaves, after which the carbon dioxide which flowed out of the space per unit of time was determined. If the quantity of carbon dioxide produced by respiration in the dark at this particular temperature is known, a simple calculation will show the quantity of photosynthesized carbon dioxide. The figures are: at 10°C, 4 mg. CO_2, at 20°C, 8,5 mg. CO_2; at 30°C, 16 mg. CO_2; at 37°C, 24 mg. CO_2; at 40°C, 12 mg. CO_2 and at 41°C, 0 mg. CO_2. From these data it was concluded that up to a temperature of 37°C the result was in conformity with VAN 'T HOFF's [263] rule that within the normal temperature range a chemical reaction is approximately doubled at every rise of 10°C in temperature.

In order to explain that above 37°C this rule no longer applies, BLACKMAN assumes that above that temperature a harmful influence sets in, which must be regarded as a limiting factor, and he correlates this research with his remarks referred to above, on the so-called limiting factors. These may be summarized by stating that in every physiological process there is always one factor which lies nearest to the minimum and that this particular factor determines the velocity of the entire process.

If this hypothesis is correct, then in the event of a gradual increase in one external factor the intensity of the process may be graphically represented by an ascending line, which takes a horizontal course as soon as this factor ceases to be limiting and another not varying factor becomes limiting. Experiments carried out by R. WILLSTÄTTER [732] and by O. H. WARBURG [699] on these designs did not seem to bear this out, they found that there were no sharp breaking points in the line representing photosynthesis. Only gradual transitions were to be observed. This gave rise to the investigations of H. HARDER [235] with the aquatic moss *Fontinalis antipyretica*. This moss does not possess any stomata, and the admission of carbon dioxide is, therefore, less complicated than in the case of higher plants. When the quantity of potassium bicarbonate dissolved in the water (which determines the concentration of carbon dioxide) and the intensity of the light are varied, photosynthesis is found to increase gradually.

$KHCO_3$	Intensity of light 667 Metercandles	2000 Mcs.	6000 Mcs.	18000 Mcs.
0.01%	0,41	0,75	0,90	1,06 CO_2
0,04%	0,91	2,24	3.45	4,70 ,,
0,16%	1,10	3,45	6,40	11,35 ,,
0,32%	1,23	4,70	8,60	15,2 ,,

The above amounts represent the sum total of the carbon dioxide which is bound in the process of photosynthesis and liberated in the respiratory process.

HARDER also failed to find any sudden changes and he, therefore, concluded that, strictly speaking, it is not possible to consider only one minimum factor. He formed the opinion that BLACKMAN's limiting factor principle was only approximately correct.

L. G. ROMELL [549] goes even further and completely denies the correctness of this principle. According to him, BLACKMAN is wrong in speaking of „factors" whilst referring to both the actual processes and the external factors by which they are influenced. The velocity of a chain of processes cannot be greater than that of its slowest link, that is a platitude; but this axiom does not imply anything as regards the factors. According to this author, therefore, there may, indeed, be limiting processes, but they must not be confused with limiting factors.

T. H. VAN DEN HONERT [267] points out that photosynthesis is a chain process. The lack of any marked transition, upon which HARDER laid so much stress, he considers of minor importance and he supposes that to a large extent this may be explained by the fact that HARDER worked with complete leaves, that is to say with complicated systems. If a leaf is illuminated from above, the chloroplasts on the upper side of the leaf receive far more light than those underneath. Hence, when the intensity of light is increased, the upper side will be the first to reach the point where light is no longer the limiting factor and therefore photosynthesis will no longer be increased. It will, however, increase in the inner layers where light still constitutes the limiting factor, thus it is never possible to observe a sudden, marked transition in multicellular organisms which do not consist of only one layer of cells. In a sense the same consideration holds good for the admission of carbon dioxide,as its diffusion by plant tissues is by no means a simple matter. Investigations by K. ZIJLSTRA [757] proved this point with regard to diffusion into the intercellular spaces of leaves. For this reason VAN DEN HONERT carried out experiments with a unicellular layer, using the filamentous Alga *Stichococcus* which floats on the substrate as a film having the thickness of only one cell.

What are the processes that can be discerned during photosynthesis? Basing his observations on the investigations by WILLSTÄTTER and by WARBURG, VAN DEN HONERT concludes there are at least three processes, the existence of which he accepts in his working hypothesis:

(1) a process of diffusion by means of which the carbon dioxide penetrates from outside into the chloroplast. This is a physical process whith a temperature coefficient of about 1,1;
(2) a photochemical process which in conformity with the law of GROTTHUS and VAN 'T HOFF, is directly proportional to the amount of light-energy absorbed;
(3) a chemical process which, according to VAN DEN HONERT, follows on the photochemical process and most likely has a Q_{10} of approximately 2, i.e. its intensity will be doubled at 10°C increase in temperature, at least up to the optimum. (so-called dark reaction)

The graph of the results obtained by VAN DEN HONERT showed that, when the photochemical process constitutes the limiting factor, there is at the point, where the line curves, only a mild deviation from the breaking point which was to be expected upon theoretical con-

siderations, and the Q_{10} then equals 1. When the temperature constitutes the limiting factor, a Q_{10} of 1,87 results between 12°C and 20°C. Lastly, when the concentration of carbon dioxide constitutes the limiting factor, the velocity of the process is governed by diffusion even in the unicellular object. The process in the chain of reactions which follows the process of diffusion is governed by the active agent having a particularly strong affinity for carbon dioxide.

The results of his work, therefore, led VAN DEN HONERT to accept the principle of limiting factors, and the deviations found by other investigators he assumes to be the result of the complicated structure of the objects used by them. However, later work by EMERSON [156] with Algae was not in line with VAN DEN HONERT's.

The experiments by WILLSTÄTTER made the existence of a chain of processes a feasible assumption. Compare, for instance, the photosynthesis of aurea-varieties with that of the normal dark-green variety of the same species. With the former it is obvious to assume that, in view of the relatively small quantity of chlorophyll, the photochemical process will act as the limiting link, and indeed a temperature coefficient of approximately 1 results with it, whereas in the latter variety this coefficient amounts to about 2, which shows that the limiting effect must be due to a purely chemical process.

Another pointer in the same direction is the fact that with a low light intensity photosynthesis is little sensitive to the effect of hydrocyanic acid, but when the light intensity is great, it is highly sensitive to it. In the last instance the so-called BLACKMAN or dark reaction is the limiting link, in which an enzymatic process activated by catalysis by heavy metals, in casu iron, plays the chief part.

(E) Influence of Light on Photosynthesis.

Matters of importance in this connection are the intensity of the light, on the one hand, and its spectral range, on the other, while the question how much of the light-energy falling on the leaf is actually utilized in the process of assimilation is a point of special interest.

In the 19th century plant physiologists used to occupy themselves particularly with the question of the intensity of photosynthesis in the various regions of the spectrum. According to SACHS and his school, there was an absorption maximum in the red region, which was supposed to be accompanied by a maximum in photosynthesis; the same opinion was held by DE VRIES. There was, however, a great deal of controversy about the existence or non-existence of a photosynthetic maximum in the blue region of the spectrum. PFEFFER [504] denied its existence, but K. A. TIMIRIAZEFF [654] argued on theoretical grounds that such a maximum did exist. It was found that pure chlorophyll has an absorption maximum in the blue region, and TH. W. ENGELMANN [157] created a stir by stating that he believed he

could confirm TIMIRIAZEFF's conclusion experimentally by means of his bacterial method. But whether this method — the demonstration of oxygen formed by virtue of the accumulation of certain bacteria — however delicate it may be, is suitable for quantitative research, remains a moot point.

For the solution of such a problem as this, it is important to find out how much of the total light-energy falling on the plant is utilized in photosynthesis. Brown [80] and F. ESCOMBE [162] experimenting with leaves of higher plants came to the conclusion that only a few per cent was utilized. Apparently much light is reflected by the surface of the leaves and absorbed in the cell-walls and the protoplasm outside the chloroplasts. PURIEWITSCH [522] attempted an ingenious solution of the problem by ascertaining how much light was absorbed by a green leaf and how much by a variegated leaf of the same species. He considered the difference between them was the amount absorbed by the chlorophyll. By assuming this to be the amount of energy used during photosynthesis, he arrived at a coefficient of utilization of 8 to 11%. But his reasoning was not altogether correct, for, apart from the chlorophyll, it was uncertain whether absorption and reflection in variegated and green leaves could be regarded as equal. Later on A. SEYBOLD [601] estimated that the value for variegated leaves might be twice that for green leaves.

O. H. WARBURG [699] and NEGELEIN [452] tackled the question of the coefficient of utilization of the light absorbed by the chlorophyll with the aid of more refined methods. By working with a suspension of the unicellular greenweed *Chlorella*, which possesses one bell-shaped chloroplast they overcame the difficulty that part of the energy is used for evaporation and that besides cells containing chlorophyll, there are also cells without any chlorophyll. In water there is, of course, no question of transpiration, while practically all the light is absorbed, provided that the suspension is sufficiently thick. The suspension was placed in a trough-shaped vessel, the inside of which had been silverpated, so that the sides should reflect what light fell upon it. WARBURG calculated the chemical energy obtained during photosynthesis from the oxygen formed, this, in turn, being determined from the increase of gas-pressure with the aid of a differential manometer. Under these conditions WARBURG arrived at a much higher coefficient of utilization, for under strong illumination it amounted to 20%, under weak illumination sometimes to about 60%. The figure varies for different regions of the spectrum and in keeping with A. EINSTEIN's [154] theoretical views it decreases in proportion to the wavelength: in the red region it is 59%, in the yellow region 53%, in the green 44%, and in the blue region 34%, under the same circumstances.

TH. SCHMUCKER [579] studied the same subject in a higher plant, *Cabomba*, one of the Ranunculaceae. He used the old method of counting the gas bubbles, which, with this aquatic plant, is possible owing

to the great regularity of the current of gas evolved. To be effective as a gauge, the number of gas bubbles should remain constant, hence it is necessary to vary the intensity of light in the different regions of the spectrum. When electric light is used, this is merely a matter of introducing resistances. The result of SCHMUCKER's experiments is very similar to that obtained by WARBURG and NEGELEIN.

E. K. GABRIELSEN [190], who worked by a different method and used seedlings of *Sinapis*, found a lower coefficient of utilization. He considers the coefficient of utilization in higher plants to be lower than that in Algae because the thallus of the species used is much thinner than the tissue of the leaves and lacks the intercellular spaces which would yield a reflexion that would produce different results for different kinds of rays. However, the results obtained by SCHMUCKER with Cabomba are hard to reconcile with this reasoning.

Recently this question was once more studied by EMERSON [156] and LEWIS [374], MANNING [405], DUGGAR [148] and co-workers; the extensive and critical research totally refutes WARBURG's results.

Reverting to the question as to whether there is a photosynthetic maximum in the blue region of the spectrum, this was denied for *Chlorella* by WARBURG and NEGELEIN, but WURMSER [746] believed he had observed such a maximum in lower organisms. HOOVER [273] came to the same conclusion with wheat. The results of more recent investigations, such as those of G. E. BRIGGS [76] and of GABRIELSEN are in keeping with those of WARBURG and NEGELEIN; for the red, green-yellow and violet regions GABRIELSEN found the ratio of 1 to 0,6 and 0,38, values which are in agreement with those found by BRIGGS. Evidently there is much controversy in this field.

The brown and red Algae were not included in these observations which exclusively concerned green plants. Much study has been given, for instance by STAHL [620], MONTFORT [439] and others to the question of photosynthesis in brown and red Algae, particularly in connection with the ecological circumstances under which they live. For example, the problem as to whether the red Algae are shadow-plants or species specially adapted to exist in the kind of light that penetrates farthest into the sea, engaged the attention of several workers. I cannot enter into this matter fully; most likely there is something to be said for either view. Later on I shall revert to the subject of the pigments of these thallophytes.

(F) Pigments of Chloroplasts.

When the third edition of the Textbook appeared E. MARCHLEWSKI [407] had just established the chemical similarity between chlorophyll and haemoglobin. The emperical formula of the two yellow pigments was known, but not their structure. It was customary to use the one term chlorophyll, although it had been pointed out by STOKES [632] as much as

30 years before then that there were two components of chlorophyll. But the chemical structure of chlorophyll was as little known as was that of the carotenoids.

About the beginning of this century TINE TAMMES [643] studied the presence of the latter substances in plants by making use of the property peculiar to carotenoids of turning blue when brought in contact with strong sulphuric acid. In the year 1906 began the chemical study of the different pigments when TSWETT [661] succeeded in separating the two green and the two yellow pigments by means of adsorption. A few years later WILLSTÄTTER [732] undertook his extensive physico-chemical research and by applying new methods succeeded in elucidating the chemical composition of chlorophyll. The same was done for the yellow pigments by P. KARRER [310]. It would lead us too far into chemical territory if we were to discuss these chemical compositions, in detail, therefore only the formulae for the two yellow and for the two green pigments are given here. β Carotene is the main constituent of the carotenoids of the leaf.

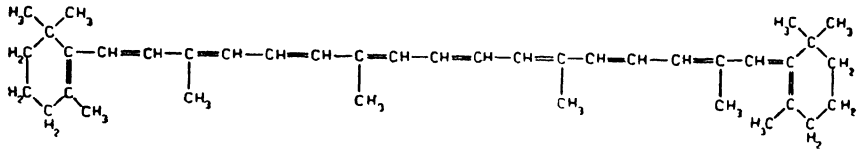


FIG. XII. β Carotene

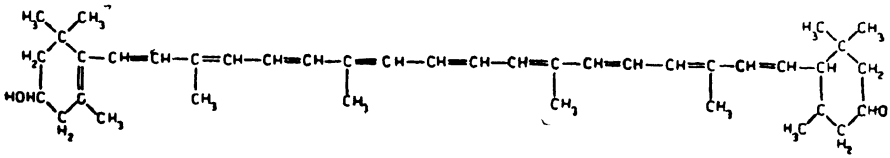


FIG. XIII. Xanthophyll

It is still undecided whether the chemical properties of the green pigments are of primary, direct importance to photosynthesis. WILLSTÄTTER formulated a theory in which the magnesium atom of chlorophyll is of significance in the binding of carbon dioxide, but nowadays preference is given to other views; for example, it is now considered that chlorophyll has the function of light sensibilisator. I shall refer to this again later, but I will only point out now that the function of the yellow pigments is still obscure. It is difficult to say, therefore, whether they have any connection at all with the process of photosynthesis. There are parts of plants which contain yellow carotenoid pigments, but no chlorophyll, whereas the reverse has never been observed in the higher plants. Of course it is clear that the yellow pigments by themselves are not capable of photosynthesis, but whether they do at all assist in the process is still uncertain.

It is only in the green sulphur bacteria that chlorophyll is found without carotenoids, and the fact that these organisms dehydrogenate hydrogensulfide into sulphur, whilst the red sulphur bacteria, which do possess carotenoids, convert sulphur further into the sulphate ion, led VAN NIEL [458] and F. M. MULLER [447] to assume that the carotenoids might have some function in this respect. However, in his recent work VAN NIEL does not hold this view anymore. A few years ago the possibility of collaboration of carotenes in photosynthesis was studied by MANNING [405].

K. NOACK [462] remarked that on the one hand chlorophyll, by virtue of its fluorescence, activates oxygen, while on the other hand the pigment is destroyed by the activated oxygen. In the living plant this does not occur, and this led him to suggest that the carotenoids, which are the first to be decomposed by the activated oxygen, might serve as a kind of protection to chlorophyll. "Se non è vero, è ben trovato"!

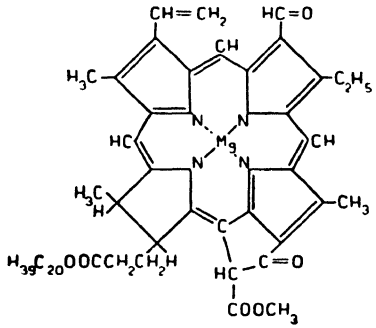


FIG. XIV. Chlorophyll a

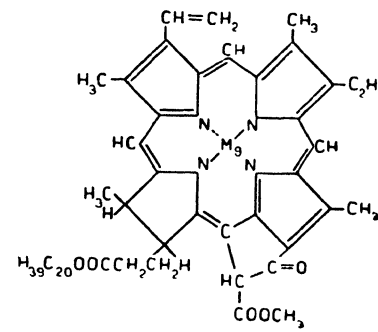


FIG. XV. Chlorophyll b

As to the green pigments, the research of WILLSTÄTTER and his collaborator A. STOLL [633] in particular shed new light upon them. As is evident from the formulae cited above, both chlorophyll A and B are phytylchlorophyllides, of which especially the 4 pyrrole groups with the magnesium atom in the centre, and the 4 CH groups, which link the pyrrole groups into a ring, are important, as are also the so-called phytyl chain and the cyclopentanone ring. The difference between chlorophyll A and chlorophyll B resides in a methyl group, contained by the former, whereas an aldehyde group is carried by the latter. Higher plants contain about 2,9 to 3 parts of chlorophyll A as against 1 part of B, but in Thallophytes there is usually more chlorophyll A whilst only in very few species more chlorophyll B is found. Red, brown and blue Algae contain practically no chlorophyll B, but in the brown Algae (Phaeophyceae) and in the Diatomeae the carotenoid fucoxanthin ($C_{40}H_{56}O_6$) is present. As early as 1884 ENGELMANN [157] credited this substance with some function in the process of photosynthesis, and this idea was recently confirmed by E. C. WASSINK [701].

Phycoerythrin and phycocyanin, respectively the red and blue pigments of the Rhodophyceae and Cyanophyceae, consist, of a protein body bound to a pigment component. Modern research has established that the phycocyanin contains 4 pyrrole nuclei in the molecule, these being situated in a row and linked together by CH groups, their structure thus showing great similarity to that of chlorophyll.

How does chlorophyll come into being? This is a question of considerable importance to physiology. From the fact that plants grown in the dark remain yellow, it was concluded that light must be essential to its formation, but with some species it seems that chlorophyll synthesis is possible even in the dark. Formerly, the fact that plants turn yellow when there is a deficiency of iron salts in the soil or in the nutrient solution was taken as an indication that this element was present in chlorophyll. When WILLSTÄTTER's work had disproved this, it was concluded by some physiologists that iron was an essential element for the formation of the pyrrole nuclei of chlorophyll. This, however, is no more than a hypothesis and its correctness still awaits proof. Another explanation of the indispensability of iron was given in the first part of this chapter.

As early as the past century, some investigators, including SACHS, spoke of a colourless precursor of chlorophyll, which was supposed to be found in etiolated plants and termed leucophyll. Chemically this substance was unknown, and it was difficult to separate it from carotenoids. During the present century NOACK [462] and KIESSLING [324] by applying modern research methods have succeeded in isolating from etiolated plants a small quantity of a substance, which they termed protochlorophyll. It was found that larger quantities could be obtained from the seedcoat of *Cucurbita* spec. These workers found that this protochlorophyll contained magnesium and pyrrole nuclei and would turn into chlorophyll under illumination. H. FISCHER [176] in his important work on the chemistry of chlorophyll considers it to be a substance with a porphyrin-ring system.

Examining the physical properties of chlorophyll somewhat closer, we have to revert briefly to its light absorbing quality. I would draw attention in particular to the place of the absorption band in the red region whose place in the living chloroplast is somewhat different from that of chlorophyll dissolved in organic solvents. But if a watery juice of chlorophyll is prepared, the band lies in practically the same place In such juice the chlorophyll is bound to protein, forming a complex termed phyllochlorine or chloroplastine. In this substance there is about 5% chlorophyll, which fact has some investigators led to conclude that 4 molecules of chlorophyll (3 of chlorophyll A, molecular weight 892, and 1 molecule of chlorophyll B, moleculear weight 906) must be present for every one molecule of protein with a molecular weight of 68,000. This would be in keeping with the condition found in haemo-

globin, where likewise 4 molecules of haematin are bound to every molecule of globin. It also fits in with the view of STOLL [633], who draws a comparison with enzymes containing a protein and a non-protein as the so-called agon.

That no photosynthesis is to be found in a suspension of the compound protein-chlorophyll might be explained by the fact that this requires a particular structure of the chloroplast. The conclusion drawn by L. G. M. BAAS BECKING [35] and his school is that chlorophyll may be present in the peripheral parts of the grana, minute particles found in the chloroplast. They consider that the phytyl chain of chlorophyll must be adsorbed to the lecithins, while the hydrophilic side i.e. the nucleus of the molecule with the magnesium atom and the 4 pyrrole rings, is bound to the protein. As the double refraction peculiar to chloroplasts indicates a stratified structure, this assumption is not improbable.

An important property of chlorophyll, which has not yet been mentioned, is its fluorescence which arises under illumination. As already stated, the maximum absorption in the chloroplast lies at about 6800 Å, and there is also a clearly observable maximum in the blue region at about 4300 Å, the same region where the maximum absorption of the carotenoids is found. Fluorescence in the living chloroplast is much weaker than in solvents and has a clearly observable maximum, which likewise lies at 6800 Å, The amount of light-energy which is radiated in the form of fluorescence, is in the living plant no more than a small fraction of the light absorbed, and recent research puts it at less than 1%.

H. KAUTSKY [315] c.s. made a study of the intensity of fluorescence during the first few moments of illumination and found that this intensity at first rises rapidly and then slowly falls to a constant level. This fall does not set in, however, when photosynthesis is inhibited by hydrocyanic acid, whilst at a temperature of 0°C. the fall is much slower than at 30°C. These facts might be explained by the assumption that this fall has some connection with the purely chemical process which is inhibited by hydrocyanic acid, whereas in the first few moments only the photochemical process occurs. Later observations by ORNSTEIN [479], KATZ [313], WASSINK [701], VERMEULEN [673] and G. REMAN [536] have, in the main, confirmed these results, although the course of events during the first moments was found to be more complicated. These investigators found that fluorescence did not rise evenly, but would first rise, then fall, and subsequently rise again; this might have some connection with the pressure of oxygen. Be this as it may, these observations on the subject of fluorescence support the view that first a photochemical process takes place, followed by one or more chemical processes. This view supports the conclusions arrived at in the discussion upon limiting links of the chain process.

(G) Theories concerning Photosynthesis.

Before discussing these theories we must mention an entirely new line of research followed in the measurement of photosynthesis. In these investigations photosynthesis was studied under momentary flashes of illumination, rapidly following one upon the other with very short intervals of darkness, the so-called intermittent illumination by flashlight.

This method was applied first by WARBURG [699], later, in 1932, also by R. EMERSON [156] and W. ARNOLD [14]. WARBURG found that, with a high light intensity and with excess of carbon dioxide, photosynthesis would increase by 100%, if intermittent illumination with a frequency of 8,000 flashes per minute was used. To explain this phenomenon he suggested two possibilities: either the reduction of carbon dioxide continues in the dark, or photosynthesis occurs more than twice as rapidly during the short moments of illumination. He considered the second possibility the more likely of the two, and he assumed that various stages of the process of photosynthesis continue in the dark until an equilibrium is reached. When after the dark period a flash of light occurs, there will be a stronger concentration of the substance already prepared. This, of course, tallies with the view that the chemical process precedes the photochemical one, a view which was expressed by WARBURG before 1924.

EMERSON and ARNOLD repeated these tests with another species of CHLORELLA and expressed the view:

(1) there is a reaction in which light-quanta must be absorbed, the so-called photochemical process;
(2) there is a reaction which occurs in the dark, the so-called BLACKMAN reaction.

When the intensity of light is very high, the photochemical reaction can occur very rapidly, and in continuous light the BLACKMAN reaction will usually have a limiting effect. The authors assumed that the BLACKMAN reaction causes the photochemical product to be converted into another and that at the same time chlorophyll is re-adapted for a new photochemical reaction. With intermittent illumination greater efficiency will result and this will be the case if each flash of light is only of sufficient duration to enable the equilibrium concentration of the intermediate product to be attained and if every dark period is sufficiently long to all of the intermediate product to be used in the BLACKMAN reaction.

In the most favourable case EMERSON and ARNOLD obtained an increase of 400%, and to obtain this result 50 flashes of light per second were required, but each flash of light was much shorter than the dark period. The duration of the dark period depends on temperature: the higher the temperature, the shorter it should be, as is to be expected in a purely chemical process. For instance, at 25°C flashes of light lasting 1/300th second will require a dark interval of 0,04 second, but at 1°C they will require a dark period of 0,4 second. Whilst the photo-

chemical process is not sensitive to rises in temperature, it is strongly inhibited by narcotics; the BLACKMAN reaction being strongly inhibited by hydrocyanic acid.

The conclusion drawn by EMERSON and ARNOLD on various grounds but more particularly based on data derived from the quantum theory, was that a large number of chlorophyll molecules co-operating as one physiological entity were required for the reduction of one molecule of carbon dioxide. During the past few years this conclusion has met with an increasingly favourable acceptance by workers in this field. H. GAFFRON [192] and K. WOHL [737] were of the opinion that these chlorophyll molecules, the number of which they put at about 2,000, must be gathered together into a particular structure, but later on it has been concluded that this is not needed. It is not necessary, therefore, to look for this physiological entity in the grana previously mentioned.

As already stated, WILLSTÄTTER in 1918 formulated a theory, according to which carbon dioxide was supposed to become bound to the magnesium atom of chlorophyll; this would give rise to carbon-dioxide-chlorophyll, which was converted into chlorophyll-formaldehyde-peroxide through the uptake of energy. Oxygen and formaldehyde being split off, chlorophyll would become available again for a new reaction. This view, which was in keeping with the old formaldehyde hypothesis of VON BAEYER [26], continued to prevail for some time and was also accepted by VAN DEN HONERT [267] in his conception of photosynthesis as a chain process. In 1933 WILLSTÄTTER to some extent adapted his hypothesis to WARBURG's view that 4 light-quanta were required for the reduction of one molecule of carbon dioxide. This mainly means that the transfer of one atom hydrogen has to be repeated 4 times, chlorophyll acting as the hydrogen carrier. Recently several American authors have accepted and used in their considerations quanta values from 10 to 12.

However, as already said above, the assumption that a molecule of carbon dioxide becomes bound to one particular chlorophyll molecule is fraught with difficulties. STOLL [633] and also J. FRANCK [180] tried to get round these difficulties by suggesting that chlorophyll bound water as well as carbon dioxide. E. A. HANSON [231] assumed that the binding of carbon dioxide to protein occurred in the form of carbamine and that the water was bound to the cyclopentanone-ring of chlorophyll. This worker also endeavoured to prove the volumetrical possibility of the assumption that chlorophyll occurs in the chloroplast in tetrads, 4 molecules together on one protein carrier of globin dimensions.

Nevertheless, there are still difficulties, particularly in connection with observations on the subject of fluorescence, and it is for this reason that the old theory of ENGELMANN [157], who said that chlorophyll played the part of a light absorbing pigment and nothing more, has again come into prominence.

In 1938 L. S. ORNSTEIN [479] and his collaborators drew the following picture of photosynthesis: chlorophyll absorbs light-energy, this brings

it into an activated state, and finally the activated state of the chlorophyll molecule attains a sufficient energetic distance from the condition at starting, the magnitude of this being the average content of the light-quanta of fluorescence-light. This state may lose its energy by being carried to an acceptor, probably water that is bound to the protein. By virtue of this uptake of a quantum of light energy, the bound water liberates hydrogen, which becomes bound to carbon dioxide. This might occur in the way as suggested by C. B. VAN NIEL [458] in 1935. Then arises:

$$CO_2 + 4H = H{-}C\begin{matrix}\diagup H\\ \diagdown O\end{matrix} + H_2O$$

Through the action of catalase the remaining hydroxyl groups will probable evolve water and free oxygen, with intermediate formation of hydrogen peroxide.

During the past few years the study of photosynthetic processes has been carried out with the introduction of isotopes. These experiments showed that the oxygen of photosynthesis comes from water and not from carbon dioxide. S. RUBEN [556] and M. D. KAMEN [308] studied the photosynthetic process with the aid of the isotope C_{11}. On the basis of their experiments they formulated the following hypothesis:

that the first stage in the processs of photosynthesis was a reversible process which was not sensitive to light: $RH + CO_2 \rightleftarrows RC\begin{matrix}\diagup O\\ \diagdown OH\end{matrix}$

that the second stage, a photochemical process, brought about with the aid of chlorophyll, converted the intermediate product $RC\begin{matrix}\diagup O\\ \diagdown OH\end{matrix}$ into $R\,CH_2OH$, this substance again playing the part had been filled by RH in the above equation, as the results of which a chain of carbohydrates was gradually formed. According to these authors, the intermediate product has a molecular weight between 1,000 and 1,600.

I must confess that I find it hard to connect these views with those discussed above.

In order not to prolong the discussion on photosynthesis unduly, I have refrained from dealing with the important investigations concerning autotrophic sulphur bacteria. In the green species of these bacteria pigments are present which are akin to chlorophyll, and in the purple bacteria there are also carotenoids. In these bacteria photosynthesis is different to this extent, that the hydrogen required for the hydrogenation of carbon dioxide may be obtained from hydrogen sulphide. Investigations into this matter by H. MOLISCH [436], J. BUDER [88], L. G. M. BAAS BECKING [35], P. A. ROELOFSEN [548] and C. B. VAN NIEL [458] to mention only a few workers of more recent times, have deepened our knowledge of the subject of photosynthesis.

However, in all these views there still remains a good deal that is entirely a matter of conjecture, and when we compare the assimilation

of carbon dioxide with the dissimilation processes we are immediately struck by the fact that during the past fifty years science has progressed much further in regard to dissimilation than in regard to photosynthesis. In my opinion the explanation may be that up to present — we cannot foretell what future development may bring to light — all observations seem to point to the fact that photosynthesis always goes with a certain structure, in this case the structure of the grana of the chloroplast. Hence, in contradistinction to respiration it has up till now proved impossible to realize photosynthesis in vitro.

(H) Products and Intensity of Photosynthesis.

In the Textbook by HUGO DE VRIES it is stated that in the reduction of carbon dioxide there is no immediate production of starch, but that first glucose is formed. This hypothesis was originally formulated by one of SACHS's pupils, A. F. W. SCHIMPER [575], and soon became generally accepted.

As we saw, DE VRIES did not mention VON BAEYER's [26] hypothesis that formaldehyde was the primary product of photosynthesis. In regard to this formaldehyde hypothesis, it must be said that, in spite of repeated attempts, it has never been irrefutably proved. In his experiments with the greenweed *Spirogyra*, BOKORNY [58] did find that in this instance the formation of starch in the dark is possible in very low concentrations of formaldehyde, but this does not prove von BAEYER's thesis. Microchemically it is also difficult to prove, and the methods of J. KLEIN [328] and WERNER [711], based on the reaction of formaldehyde with dimedon or ureum, are open to many objections.

WILLSTÄTTER [732] succeeded in proving that under all circumstances the photosynthesis quotient $\frac{O_2}{CO_2}$ is invariably equal to 1. From this it follows that the product formed in photosynthesis must have the formula $C_p H_{2n} O_n$. Among the various products that may possibly be formed, formaldehyde is the simplest, but glycol aldehyde has the same general formula and this compound has also been named as the primary product. There is, moreover, a possibility — and this is assumed by RUBEN [556] and KAMEN [308] — that a complicated carbohydrate is gradually evolved.

SCHIMPER's theory, that glucose, being the primary carbohydrate formed, was of first rate importance, was challenged in 1893 by H. TH. BROWN [80] and D. MORRIS [440]. From their observations of the carbohydrate quantities in the leaf of *Tropaeolum majus* in the morning and in the evening, these two investigators concluded that not glucose, but sucrose was the primary carbohydrate formed. In 1898 F. A. F. C. WENT [709] concluded from his experiments on sugar cane, *Saccharum officinarum*, that the primary product in this object likewise was sucrose. The main argument of BROWN and MORRIS was that during the day

the quantity of sucrose per surface unit gradually increased in the course of photosynthesis and decreased during the night. Glucose behaves quite differently, for it is always present in a relatively small quantity. But this result might also be explained by the assumption that the carbohydrate primarily formed in photosynthesis is, actually, glucose, but is converted into a diose as and when it arises.

In 1923 the present writer succeeded in proving the correctness of this assumption. If the leaves of a variegated variety of *Pelargonium zonale* were rid of their carbohydrates by putting them a few days in the dark it was found that immediately after their resumption of photosynthesis the presence first of monoses could be demonstrated in the still undamaged leaves. Next dioses (sucrose) were found to be present, and still later starch.

M. C. KEULEMANS [321] confirmed this conclusion by his experiments on *Tropaeolum majus*. An additional indication is the fact that in variegated leaves the yellow parts, in which no assimilation can occur, contain the diose, sucrose, but no monoses, whereas in the green parts of such leaves both kinds of carbohydrates are present during photosynthesis. It is still undecided whether the product primarily formed is glucose or fructose.

Experimenting with leaves of *Helianthus annuus* J. H. C. SMITH [613] came to a different conclusion. There is a temperature effect on the proportion of different classes of carbohydrates recovered after short periods of photosynthesis. This effect apparently favours the accumulation of disaccharides such as sucrose at lower temperatures. The carbon content of the material accumulated during photosynthesis approaches that of a disaccharide. The latter reminds us of the result of BROWN and MORRIS and may perhaps be explained in the same way.

From what has been said it is clear that formation of starch begins when the concentrations of sugars are already on the increase. There is a good deal of literature on this subject of conversion of sugars into starch and vice versa, as also on the differences between the so-called sugar-plants and starch-plants, of which the former particularly accumulate sugars, the latter starch in their leaves. However, in most cases the stomata cells of the leaves of the sugar-plants do contain starch, a subject already referred to in Chapter IV.

It is not possible to discuss all this here. In recent years important work concerning the above mentioned interconversion of sugars was done by C. HARTT [242].

The subject has a totally different aspect since 1936 when HANES [229] studied glycolysis. The relation of sucrose to starch has found an explanation in the action of the phosphorylases catalysing the reversible actions of:

starch $\rightleftarrows$ glucose-1-phosphate and glucose-1-phosphate $\rightleftarrows$ sucrose.

A conversion as for instance takes place during a few days in the ripening

bananas when starch disappears and sucrose arises may be explained in this way. A phosphorylation theory in photosynthesis has been proposed by LIPMANN [383] c.s.

SACHS [565] and various later authors collected data in the subject of intensity of photosynthesis, but there is no point in mentioning these in this context. Since 1904, when BLACKMAN [52] introduced the principle of limiting factors, or, to quote ROMELL [549], of limiting links, absolute values have lost much of their significance. All that can be done is to ascertain which is the external factor that should be regarded as the limiting one. In general, the carbon dioxide content of the atmosphere is found to have a limiting effect, but in this connection a distinction should be made between sun plants and shadow plants. Among the many investigations into this matter I should mention the work done by P. BOYSEN JENSEN [69] and the data collected by him and his pupils at Copenhagen.

BOYSEN JENSEN compares a sun-plant such as *Sinapis alba* with a shadow-plant, e.g. *Oxalis actosella*. The former grows in the open field, where according to BOYSEN JENSEN's observations, the intensity of light on a summer's noon may be 50,000 Lux, at least at the latitude of Copenhagen. The latter grows under beech-trees, where the intensity of light sometimes amounts to no more than 1% of this value, i.e. 500 Lux. The so-called compensation point, where photosynthesis and respiration balance each other, lies in the case of the shadow plant, *Oxalis acetosella*, woodsorrel, at a much lower intensity of light than in the case of the sun-plant *Sinapis*, but under natural conditions the optimum of photosynthesis lies much higher in the latter plant. For the sake of precision, a few figures are mentioned here: the optimal photosynthesis of *Sinapis alba* occurs at an illumination of about 12,000 Lux, that of *Oxalis acetosella* at about 2,000 Lux. Under such conditions the photosynthesis of the former per hour and per 50 square cm. of leaf surface, with a carbon dioxide content of 0,03% and a temperature of 20°C, amounts to 10—12 mg. CO_2. In the same circumstances though with an illumination optimum of 2000 Lux the amount of carbon dioxide bound by *Oxalis acetosella* is no more than 1 mg.

As already stated in connection with the red and brown Algae, Rhodophyceae and Phaeophyceae, submerged waterplants may be regarded more or less as shadow-plants. Owing to their environment, submerged waterplants belonging to the Phanerogams show many anatomical pecularities. For instance, they lack stomata, so that the carbon dioxide, which is dissolved in the water, has to diffuse through the cuticle which is but little differentiated. Besides carbon dioxide, however, calcium carbonate and calcium bicarbonate with their ions are nearly always present in water, and the p_H determines the mutual ratio of these ions.

The view usually taken is that during photosynthesis the carbon dioxide that has diffused inwardly supplies the material for photo-

synthesis, but K. ARENS [14] suggested another possibility. He assumed that, as a result of an inherent polarity of the plant, a polar transmission might take place, in the course of which all the components of the $Ca(HCO_3)_2$ solution were taken up through the lower surface of the leaves and at the upper surface a $Ca(OH)_2$ solution was liberated, which in the presence of carbon dioxide would precipitate on the upper surface of the leaves in the form of calcium carbonate. As ARENS leaves the cause of this assumed inherent polarity totally unexplained, its existence is uncertain.

(I) Assimilation of Nitrogen and Formation of Proteins.

In the Textbook this subject receives but scanty consideration; yet this lacuna cannot be attributed wholly to the time when the book appeared, for in PFEFFER's book "Plantphysiology" [504] which appeared only a few years later, much more is said about it.

Ever since J. B. D. BOUSSINGAULT's [67] work it was known that the majority of autotrophic plants can use nitrogen only in the form of nitrates or ammonium compounds, but very little had been ascertained concerning the assimilation of these substances and the formation of proteins. A few authors, e.g. PFEFFER and SCHULZE [441] had, indeed, pointed out the importance of amides, particularly of asparagine, but no deeper insight had been attained at the time. Moreover, a serious obstacle was the general lack of knowledge as regards the structure of the protein molecule. The research by E. FISCHER [175] and others, as a result of which this molecule has come to be regarded as a polypeptide formed by chains of connected amino acid residues, dates only from the 20th century.

The uptake of salts by the roots has already been dealt with, so that we can now proceed to the question as to whether nitrates and ammonium compounds are of equal value to the higher plant. For the large majority of plants, nitrites are poisonous, even in small quantities and low concentrations.

In connection with the question which is more useful the NO_3 anion or the NH_4 cation, it should be stated that normally a process of nitrification takes place in the soil, which converts ammonia into nitrates. This is the more important because the quantity of N- compounds in the soil is extremely small.

It will be seen readily that consequently the quantity of nitrogen bound will in many cases constitute the limiting factor in the process of growth. Hence the custom of using manure, which contains bound nitrogen, dates back to remote antiquity. In the soil only a few per cent of the small quantity of nitrogen is present in the form of nitrate — or ammonium nitrogen —, the remainder being found in the proteins of dead or living bacteria. Ammonia is gradually liberated upon the death of the bacteria.

By 1877 SCHLOESING[577] and MÜNTZ[446] had established experimentally that chloroform stopped nitrification, but it was S. WINOGRADSKY[735] who succeeded in isolating the bacteria which carry out this conversion. It was found that in this process two stages could be distinguished: first the ammonia is converted into nitrous acid by *Nitrosomonas europaeus*:

$$NH_3 + 3O = HNO_2 + H_2O + 70{,}000 \text{ cal.}$$

The nitrous acid thus formed is subsequently oxidized into nitric acid by *Nitrobacter Winogradskyi:*

$$HNO_2 + O = HNO_3 + 21{,}600 \text{ cal.}$$

From a physiological viewpoint it is interesting that owing to the energy obtained in these processes the bacteria are capable of reducing carbon dioxide. In contradistinction to the phosynthesis of green plants with the aid of sunlight energy, this is a case of chemosynthesis. Hence these bacteria possess a chemo-autotrophic metabolism, a phenomenon also encountered with the sulphur and iron bacteria, as was explained by WINOGRADSKY in 1887. The ratio of the nitrogen converted in the process of nitrification to the carbon assimilated is 35 : 1 in the case of *Nitrosomonas*; in Nitrobacter the ratio is far less favourable, owing to the smaller caloric output: for every 100 molecules of nitrogen converted hardly one molecule of carbon is reduced.

Likewise curious is the discovery that different kinds of organic substances, such as glucose, urea and asparagine, proved a serious impediment to the development of *Nitrosomonas*. Most of these compounds also have an inhibitory effect on its respiration, as was found by MEYERHOF[427], though in the presence of glucose this is not so. T. Y. KINGMA BOLTJES[325], however, stated that certain organic preparations containing proteins had a favourable effect on the development of *Nitrosomonas*, but when ammonia is not present, no development occurs, so that in that case nitrification remains indispensable. The development of *Nitrobacter* is likewise inhibited by organic substances, and also by ammonia or ammonium compounds.

In nature it is undoubtedly a matter of importance that there should be gradual transformation of ammonium compounds into nitrates, for the latter are easily washed from the soil by rain; as it is, they are taken up by the roots as soon as they are evolved.

J. H. QUASTEL[523] has carried out experiments on the mode of transformation of nitrogen compounds into nitrate in the soil. The interpretation of the results is that the nitrifying bacteria grow in the surface of the soil crumbs at the sites where ammonium ions are held in base-exchange combinations and proliferate at the expense of such adsorbed ammonium ions.

In 1895 the general inclination was to follow BOUSSINGAULT'S[67] lead and to regard nitrates as the most important sources of nitrogen, although

as early as 1863 HELLRIEGEL [248] had pointed out the significance of ammonium compounds. It is probable for this reason that HUGO DE VRIES, whose agricultural work had brought him into contact with actual practice, refers to nitrates and ammonium compounds as being of equal value.

Extensive research carried out with different agricultural plants in 1900 once more convinced P. WAGNER [692] that nitrates were to be preferred. Nevertheless, even in his experiments the possibility of nitrification was not excluded, and it was not until PITSCH [511] sterilized the soil used that this difficultywas overcome. PITSCH conclusion was that under these conditions nitrates were preferable, though it was found later that for a few plants e.g. barley, oats and mustard both products might be regarded as of equal value.

The less favourable results obtained with ammonium salts were explained by (1) the strong production of ammonia owing to the presence of calcium compounds; (2) the strong adsorption of ammonia to silicates; (3) the rapid uptake by bacteria. But these explanations could hardly be said to hold good in the case of water cultures, where, generally, nitrates were also more effective. As early as 1881 AD. MAYER [417] formulated his theory of physiologically acid or alkaline nutrient salts. Thus ammonium sulphate may be called physiologically acid. As already stated in the discussion on salt intake, the anion is sometimes not taken up from a solution to the same extent as is the cation, and an exchange of the cation against H ions will lead to an acid reaction in the solution. In connection with these facts D. N. PRIANISCHNIKOW [518] asserted that ammonium nitrate also is an physiologically acid salt. In this case the NH_4-kation is taken up more rapidly than the NO_3-anion. W. MEVIUS [423] was the first to attack the problem experimentally. In his tests with water cultures the action of the ammonium salts was found to depend entirely upon the p_H of the solution. With a neutral or weakly alkaline reaction these salts are a poor source of nitrogen, even poisonous. Growth is inhibited, the roots become glassy and die; the higher the p_H, the stronger is their poisonous effect. With a p_H of 5,3—5,6, the ammonium salt is as good a source of nitrogen as the nitrate. When growing conditions were good, with a p_H of 5,3—5,6, MEVIUS observed no change in the degree of acidity as a result of the use of ammonium nitrate. He was inclined to look for an explanation in the fact, already noticed by HUGO DE VRIES in 1871, that ammonia in a watery solution penetrates very rapidly into the vacuole. Modern research shows that this is a case of a high degree of permeability to the undissociated molecule, though not to the OH-ion, as can be seen from a comparison with the influence of a diluted solution of potassium hydroxide.

The question is whether this explanation by MEVIUS, though in itself quite logical, accounts for all the facts. Perhaps the matter is more complicated, especially if we consider the investigations by VAN DER HONERT [267], mentioned previously, relating to the uptake of

phosphates by the roots of sugar cane, which in an environment of constant composition, was found to be at its maximum with a p_H of about 4.

What is the fate of nitrates and ammonium compounds taken up? Is it possible for us to follow them on their way and to see where their further assimilation occurs? SCHIMPER [575] had already determined the fate of the nitrates. With the aid of diphenylamine it is possible to follow them as far as the vascular bundles of the leaves, but they disappear in the mesophyll, as can be observed particularly well in etiolated plants.

Because of the various objections that can be raised against the reaction with diphenylamine, e.g. that it is useless when aldehydes are to be found in the tissue, the present writer also applied SCHLOESING'S method, by which the nitrates are reduced to nitric oxide and then determined volumetrically. The result confirmed SCHIMPER'S opinion: in the vascular bundles of etiolated sugarbeet a fair amount of nitrate is present, but it is nearly lacking in the mesophyll. With *Pelargonium zonale*, the normal green leaf of which usually yields no reaction, or at best a very weak one with diphenylamine, it can be called forth by keeping the leaves in the dark for some time, only to disappear again under illumination, though more rapidly from green leaves than from variegated ones. From these facts it may be concluded that the nitrates are utilized for the formation of other nitrogen compounds as soon as the other requisite material is present. It will be seen later whether or not light is directly concerned in this process.

SOPHIA ECKERSON [153] made tests with tomato plants. Plants grown in a complete nutrient-solution were transferred to a solution from which nitrogen compounds were absent. Under such conditions the nitrates disappear from the leaves, and when after a while the plants were transferred back to a normal nutrient-solution, it is possible to demonstrate the presence of these nitrogen compounds, first in the roots and later in the leaves. Miss ECKERSON believed she found that subsequently nitrites, next ammonium compounds and finally amino acids arose in the leaves, but the methods she employed are open to much criticism.

When buckwheat, *Polygonum Fagopyrum* is grown in a water culture without ammonium compounds, though containing nitrates, the ammonium compounds will be lacking in the roots, but will be present in the leaves; apparently they have been formed there from the nitrates taken up. The present writer found that the microchemical reaction to ammonium compounds was somewhat stronger in etiolated and in variegated leaves than in green leaves of the same species. This seems to indicate that their assimilation has some connection with photosynthesis or to put it more precisely, with products formed in connection with photosynthesis.

It has also been found possible to effect assimilation of the nitrates taken up in isolated roots, provided that such roots contained a sufficient quantity of carbohydrates. Tests of this kind, which were carried out

by W. POSTMA [516] in the writer's laboratory, showed that an abnormally strong respiration then took place, a fact which fits in with the experiments by WARBURG [699] and NEGELEIN [452] on *Chlorella*, to which reference has already been made. When nitrates are used as a source of nitrogen, this Alga produces an abundance of ammonia if the rest of the nutrient-solution is normal. Simultaneously with this formation of ammonia there is an abundant production of carbon dioxide. In relation to this, WARBURG spoke of extra-carbon dioxide, which he took to be the result of the oxygen liberated in the reduction of nitrates.

WARBURG at the same time considered the question whether light has any influence on this reduction of nitrates. A difficulty is that in light there is also carbon dioxide reduction as soon as there is photosynthesis, so that the additional carbon dioxide is re-assimilated. However, since in light more additional oxygen is produced than would correspond with the additional carbon dioxide produced in the dark, WARBURG concluded that the question must be answered in the affirmative. This view is supported by results obtained with narcosis by means of phenylurethane, which in the concentration used almost completely inhibits carbon dioxide reduction and decreases the nitrate reduction by 30% at the most. During the action of the urethane the amount of additional carbon dioxide production in the light is more than twice that produced in the dark. According to WARBURG this indicates an enhanced nitrate reduction in the light, but it may also be due to an increased uptake of nitrates.

We have now arrived at the problem of protein synthesis, which is analogous to carbohydrate synthesis. Both these problems have engaged the attention of plant physiologists for the past fifty years, and rightly so, for just as the carbohydrate-nutrition of animals and heterotrophic plants depends, in the last instance, upon photosynthesis in the parts containing chlorophyll, so the nitrogen nutrition of humans and animals is dependent upon proteins derived directly or indirectly, from plants.

Owing to the prevailing lack of knowledge concerning the chemistry of proteins plant physiologists for a number of years had to confine themselves to vague generalities and could only study the circumstances under which the process occurs. For instance, in 1903 F. LAURENT [365] and E. MARCHAL [406] argued that light, particularly of short wave-lengths, favoured the formation of proteins though their methods were unsatisfactory and their results accordingly not conclusive. They were opposed by W. ZALESKI [750], who insisted that in any case it is not always correct to say that light is essential. He based his view on the protein synthesis in onion bulbs, *Allium Cepa*, which can occur in darkness. This however, is not altogether comparable with protein synthesis in leaves, because in the latter case the protein synthesis starts from nitrates or ammonium compounds, whereas in the onion the synthesis starts from amino acids.

U. SUZUKI [638] found that in etiolated barly seedlings protein was formed

from nitrates and sucrose, which were taken up in the dark. Hence the synthesis of proteins in the dark is possible, but it is still undecided whether light accelerates the process. During the next few years various investigators argued that, when a plant, or part of a plant is placed in the dark, a proteolysis, breakdown of proteins sets in and that this process can be inhibited by the presence of carbohydrates.

At that time very little was known of protein metabolism in the green leaves. In the Textbook by BENECKE [38] and Jost [305] of 1924 we even find the following curious method given to investigate whether proteins move out of leaves during the night. T. KOSUTANY [345] had found that at night the quantity of protein contained in vine leaves decreased, if calculated on a basis of dry weight. BENECKE soon realised that KOSUTANY's method of calculation was incorrect and should have been based on the leaf surface, but because these data were not available, he took the ratio of dry weight to leaf surface of a totally different species and applied his figures to KOSUTANY's results. This method is, of course, entirely inadmissible.

The gap in our knowledge was filled by the work of CORNELIA A. GOUWENTAK [207], who studied the metabolism of *Helianthus annuus* in the author's laboratory. She found that both synthesis and breakdown of proteins occur in the leaves, and that sometimes a removal to the stem may be observed. During the morning hours synthesis outweighs the quantities translocated or broken down, but later in the day the reverse is the case. Thus it may happen that at the end of the day the total quantity has remained unchanged, but if wilting has caused the synthesis to become inhibited or if after the plant has flowered there is a strong decrease in proteins through translocation in some form or other, the total quantity in the leaf will be less at the end of the day. Sometimes a decrease is observed overnight, at other times the quantity of proteins remains equal. Later investigations of WALKLEY [695] and others have shown that in leaves the capacity for synthesis decreases with age although in exceptional circumstances the process remains possible and shows little or no decrease.

At this date ($\pm$ 1930) all research workers in this field agreed that the following factors had to be regarded as the most important in the synthesis of proteins: (1) the presence of the products of photosynthesis; (2) the presence of nitrates or ammonium compounds, in addition to which the watercontent was thought to exert some influence.

In 1935 K. MOTHES [442] introduced a new factor by stating that the oxidation-reduction potential, i.e. the oxygen tension, was the determining element in protein metabolism. In his observations, which were strongly influenced by the work of E. WALDSCHMITZ-LEITZ[694], MOTHES adopted the point of view already mentioned, that the synthesis and breakdown of protein occur simultaneously and that thereby the dynamic equilibrium of these two processes is shifted to either one side or the other, due to the activation or inhibition of special substances acting

as regulators upon the enzyme actions. He regarded papain as a special enzyme of protein metabolism. This substance affects iso-electric protein and is activated by hydrocyanic acid and by SH or sulphhydril groups. The natural activator might be a substance with a sulphhydril group, such as glutathione, which is the cause of a decomposition of protein at a p_H of 6,8 and an oxidation-reduction potential of less than 300 millivolts. At a potential of 400-550 millivolts occurs the reverse and a synthesis of protein is promoted. Substances which reduce the S—S group of cystine, give rise to the SH-group and are thus capable of being activators, but when oxygen tension is increased the SH-group is broken down and the proteolytic action of the papain decreases.

MOTHES observed that the papain content in young leaves differed from that of old ones, a fact which cannot be directly determined by autolysis. The younger parts do have a higher concentration of the enzyme, but this is only partially activated, whereas in old leaves the quantity, though smaller, is entirely activated. When young parts are placed in an environment with a low oxygen tension, the action of papain sometimes becomes ten times as strong, but with old leaves under these conditions hardly any increase is to be observed.

Resting seeds contain hardly any papain, not even when placed in an environment that is poor in oxygen. Germinating seeds, however, do contain the enzyme, which becomes much more strongly activated during germination.

According to MOTHES there is also much protein synthesis in tissues with strong respiration. This, in his opinion, has no direct bearing on the energy obtained in respiration, but occurs by virtue of the oxidation of the activator, as a result of which proteolysis decreases. It is assumed that there is a daily rhytm of protein synthesis in green parts. This is considered to be dependent not on the carbohydrate content, but on the oxygen tension, which is increased through photosynthesis in the daytime. Thus, in relation to the tension and potential of oxygen, light has an indirect influence. The closure of stomata in the dark is also of importance; according to MOTHES, the apparent inhibition of protein synthesis by wilting is entirely due to the fact that the closing of the stomata causes a decrease of the oxygen tension in non-photosynthesizing parts.

MOTHES also made some interesting injection experiments. Some leaves were evacuated under the receiver of an air-pump and subsequently the intercellular spaces were filled with ammonium-lactate. At first a breakdown of proteins prevailed, but as soon as the intercellular spaces became filled again with air, synthesis got the upper hand, a phenomenon which must surely be associated with the enhanced oxygen potential.

Generally speaking, it may be said that MOTHES's views are fascinating in that they co-ordinate respiration, transpiration, photosyn-

thesis and protein metabolism through their connection with oxygen potential and thus give greater prominence to the unity of the metabolism of the living organism. Yet this fascination by no means implies that his views are correct from beginning to end. One weak point is that his experiments only serve to explain the influence of the oxygen tension on the breakdown of protein; protein synthesis more or less takes a secondary place. The difficulty is that, although it is possible to inhibit synthesis by means of narcotics whilst allowing dissimilation to continue, we are still ignorant of the means to achieve the reverse process.

It stands to reason that such sweeping views were bound to meet with criticism. The chief critic was K. PAECH [492], whose main argument was that MOTHES only studied post-mortal processes in his experiments, whereas in the living tissue the oxygen tension has a totally different effect. This may be partially correct, but is certainly not so in all cases, for in the protein synthesis in the onion bulb the oxygen potential does play a part.

Moreover, PAECH has very little to say that is new: that the synthesis or hydrolysis of proteins is dependent upon the quantity of chemically active carbohydrates and nitrogen compounds means very little and is even not correct in some instances. CHIBNALL [110] points out the weakness of PAECH's assumption that the active mass of monoses determines the active mass of α ketonic acids, the probable precursors of amino acids.

His incorrectness is also evident from the work of J. KABOS [306] in his study upon the metabolism of nitrogen in seedlings of *Sinapis alba*. In his experiments KABOS to the best of his ability avoided features of the above mentioned investigations by LAURENT [365] and MARCHAL [406] which had come in for fairly widespread criticism. For instance KABOS worked with seedlings that had arrived at a stage of germination where the reserve proteins were found to have been decomposed to a large extent and synthesis was beginning to preponderate. With such material he attempted to test the theory of MOTHES as well as that of PAECH. As already stated, in several cases PAECH's views were found to be incorrect, but also the oxygen tension was observed to have little effect. KABOS's experiments under these particular conditions were able to show that sunlight was not of direct importance to protein synthesis, though he found that ultra-violet light between 3900 and 4300 Å had a favourable influence.

So far, only the general circumstances under which protein synthesis occurs have been considered and the more chemical side of the problem concerning the ultimate production of protein from ammonium compounds in combination with other substances must be reviewed. But first it should be mentioned that according to BURSTRÖM [99] the leaves of *Triticum vulgare* cannot use the carbohydrates supplied to them; photosynthesis seems here to be indispensable for protein synthesis.

Following M. GRESHOFF's [212] investigations with the tropical tree

Pangium edule, M. TREUB [657], Director of the Botanical Gardens at Buitenzorg made physiological experiments with *Phaseolus lunatus* and in 1896 formulated the hypothesis that hydrocyanic acid was the first stage in protein synthesis. A few years later H. FRANZEN [183] formulated a purely chemical theory in this respect. He considered that by virtue of the action of formaldehyde on nitrates, hydrocyanic acid was formed via ammonia, and the combination of one molecule of hydrocyanic acid with one molecule of ammonia and one molecule of formaldehyde gave rise to aminonitrile which in combination with water may form glycine,

$$CH_2\ (HN_2)\ COOH.$$

But this elaborate synthesis of an aminoacid met with little approval, nor was TREUB's physiological view generally accepted.

Investigations by ED. VERSCHAFFELT [675] and subsequently by L. ROSENTHALER [553] and by N. J. STEKELENBURG [624] showed that TREUB's arguments could not be substantiated. In view of physiological experiments on a number of plants containing hydrocyanic-acid-glucosides, STEKELENBURG argued that hydrocyanic acid does not arise directly from nitrates, but is formed as a by-product of protein metabolism. Generally speaking hydrocyancic acid does not occur in the plant in a labile form, but is bound in the form of a glucoside serving as a reserve substance.

While the TREUB-FRANZEN theory is now only of historical interest, the following conception regarding the formation of amino acids is more attractive. As a results of the work of F. KNOOP [336] and H. OESTERLEIN [470], it has during the past few years become extremely probable that this formation occurs through the action of ammonia on ketonic acids. As was clearly shown by A. J. KLUIJVER [331] in his biochemical observations, there is a series of voluntary reactions of the type $AH + B \rightarrow A + BH$. By "voluntary reactions" is meant that, taken as a whole, the free energy decreases, although the energy content of BH may be greater than that of B.

Thus the scheme for the synthesis of amino acids from a monose and ammonia will be as follows: in the dissimilation of the sugars a ketonic acid is formed, pyruvic acid (which is represented in the scheme by R. C. = O.COOH):

$$\text{I}\quad R.C. = O.COOH + NH_3 \rightarrow R.C. \begin{matrix} OH \\ NH_2 \end{matrix} COOH$$

$$\text{II}\quad R.C. \begin{matrix} OH \\ NH_2 \end{matrix} COOH \rightarrow R.C. = NH.\ COOH + H_2O$$

$$\text{III}\quad R.C. = NH.\ COOH + 2\ H \rightarrow R.C.HNH_2COOH \text{ (amino acid)}$$

The hydrogenation in III must be considered as linked to the dehydrogenation which occurs in the dissimilation of sugar.

$$C_6H_{12}O_6 \rightarrow 2\ CH_3COCOOH + 2\ H$$

Once an amino acid has been formed in this way others may arise

from this by transamination, a process studied by A. E. BRAUNSTEIN [72] and M. G. KRITZMANN [350]. In the presence of a dicarboxylic acid any amino acid may react with any ketonic acid by a partial exchange of the CH (NH_2) group for the ketogroup. As an example the conversion of glutamic and pyruvic acid is given here as follows:

$$\begin{array}{c} COOH.CH_2.CH_2.CH(NH_2).COOH + CH_3CO.COOH \rightleftarrows \\ COOH.CH_2.CH_2.CO.COOH + CH_3CH(NH_2)COOH \end{array}$$

Acid amides cannot carry out this conversion, but amino acids can do so, and it appears that the reaction between pyruvic acid, on the one hand, and aspartic acid on the other is the swiftest. I shall refer to this again later when discussing the breakdown of reserve proteins. I should point out, however, that in seedlings an enzyme system has been found, which causes such transamination to occur fairly rapidly at ordinary temperature, It is also noteworthy that the part played by the ketonic acids in this synthesis of amino acid indicates that there is a relation between protein synthesis and dissimilation; to this also I shall refer later on.

Another interesting point is that in the experiments of both MOTHES and KABOS ammonium lactate was found to be a substance which was particularly suitable for the formation of proteins, lactic acid being closely related to the products of carbohydrate dissimilation.

Thus, with due caution, it may be said that there is every reason to assume that protein synthesis occurs via amino acids, an assumption which agrees with the fact that, by linking the residues of amino acids in vitro, chemical compounds have been obtained which are termed polypeptides and which may be regarded as artificial proteins by virtue of their reactions.

It has been established that in the hydrolytic breakdown of proteins about a score of amino acids are formed. It is not necessary to mention all of them here by name; they are to be found in every book of organic chemistry. Now the question arises whether it has to be assumed that all these amino acids are first pre-formed to be subsequently built up to actual protein. It has not yet been possible to demonstrate the presence of these amino acids in the places where protein synthesis may be expected to occur, such as the growing points, but notwithstanding this there is every reason to believe that these amino acids are pre-formed in the plant, at least in the higher plants.

The transamination just referred to does not solve the problem, because there are a number of amino acids with an entirely different structure, e.g. cystine, proline and tryptophane.

The reason for the belief that amino acids are preformed is this: alkaloids are complicated nitrogenous organic substances, the occurrence of which, but for a few exceptions, is confined to the higher plants. Generally their structure to some extent resembles some amino acid

or other. By splitting carbon dioxide from a carboxylic group, the so-called decarboxylation, or by methylation or ring closure, various alkaloids may arise from amino acids, for instance the alkaloid hydrastine from dioxyphenylalanine, or the ergot alkaloids from tryptophane. Particularly in the case of alkaloids such as caffeine, which occur in different families of plants that are not closely allied, this relation is a simple one: for example, caffeine = trimethylxanthine, xanthine itself being a product of the decomposition of the nucleoproteins which are present in every cell.

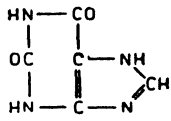


FIG. XVI. Xanthine

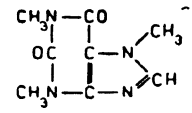


FIG. XVII. Caffeine

The present writer found that alkaloids are generally formed in the growing points, i.e. the places where protein synthesis occurs. Hence the presence of some alkaloid or other may be taken as an indication that the substance from which it arose, in this case some amino acid, is also present in these growing points.

To conclude this discussion on protein synthesis in the higher plants, I wish to go somewhat deeper into work by T. C. STEWARD [629] and G. PRESTON [517] of 1940. They made a balance of the metabolism of the object which the first-named author studied repeatedly, viz, thin slices of potato. The dry weight, uptake of anions and cations, respiration, content of starch, sugars, protein-nitrogen and soluble nitrogen, combustion value, all these data were determined. In short, a complete balance was drawn, as has seldom been done for botanical objects and never under the circumstances described. The slices were kept for 70 hours either in distilled water or in solutions of potassium bromide, calcium bromide or potassium nitrate of varying concentrations, the oxygen tension also being varied.

This experiment, which took up a good deal of time, revealed much that was of interest regarding various details of metabolism, particularly because the account balanced fairly well. So I will first say something about those processes which, to all appearances, have little to do with protein synthesis. It is typical that this protein metabolism is actually found to be linked to a number of other processes, or, in the words of the authors: "The results suggest that aerobic respiration, protein synthesis, water absorption and salt accumulation are all actually dependent processes, which occur in cells which are not subject to equilibrium conditions, but the behaviour of which, at constant temperature, is regulated by oxygen tension and the nature and concentration of the salt solution". This view of their being mutually dependent — which reminds us of the views of MOTHES [442] — has a great attraction for the present writer,

because it tends to penetrate further into the real nature of metabolism. Up to now the aim has always been to try and study each process separately, yet the living protoplast regulating these processes is a unit not to mention the individuality of the plant. Even though sometimes a detail of the whole can be reproduced in vitro with the aid of certain enzyme complexes, the whole itself is not so realizable.

Entirely in keeping with what has already been discussed, it was found by STEWARD and PRESTON that in this connection also oxygen is essential for the intake of ions. Water absorption, far from being a purely osmotic process, must also be regarded as a vital process, which is linked to respiration. It is curious that the authors noted a very faint respiration in the solutions of calcium bromide, weaker than in distilled water. Since in those solutions bromine ions are taken up, the authors here contest the accuracy of LUNDEGARDH's [393] formula concerning the basal respiration mentioned above:

$$R_t = R_g + k.A$$

In relation to protein synthesis, it was found that when slices of potato were placed in distilled water, this synthesis occurred at the expense of amino acids. The authors further noted that controls placed in a calcium bromide solution and having the least water absorption and the weakest respiration, also had the least protein synthesis. Similarly, controls in the potassium nitrate solution, having the greatest intake of water and the strongest respiration, showed the greatest protein synthesis.

Several investigators have demonstrated that in the aerobiotic oxidase system of the potato an ortho-quinone plays a part, and this system is capable of disaminating alanine, phenylalanine and other amino acids. STEWARD and PRESTON showed that in the case of their slices of potato, treatment with inorganic salts and admission of oxygen, which influence respiration as well as protein synthesis, also have a regulatory effect on the activity of this oxidase system. The quantity of carbon dioxide liberated during the synthesis of proteins shows, moreover, that the residue of carbon, which remains after disamination of the amino acids, can be responsible only for a small part of the carbon dioxide formed. This means that both sugars and amino acids have the same relation to respiration and protein synthesis. Though these two groups of substances are the sources of carbon and nitrogen required for the synthesis, their concentration does not regulate respiration; their consumption is determined by the action of the oxidizing system, which in its turn is regulated by the salts present and the oxygen potential.

WOOD [741] and PETRIE [503] using differential treatments of sucrose and ammonium salts on *Phalaris* plants found a high negative correlation of protein on sucrose and glucose content, whilst the relation on fructose was insignificant. Nor could they find a significant correla-

tion of protein on any function of the ammonium content. On the other hand the variance of protein content could be almost wholly accounted for in terms of the amino acid and water contents all expressed in a dry weight basis. WOOD's conclusion is that the above mentioned hypothesis of PAECH is untenable and that proteins are probably formed from the whole of the amino acids rather than along any alternative path. However, the relations between protein and total amino acid concentration are still obscure. PETRIE and WOOD suggest that there is some other limiting factor. Interrelation between respiratory rate, protein and amino acids could be best explained by the hypothesis that respiration increases as the amino acid concentration increases and that some amino acids are more readily disaminated than others.

Thus the connection between these different processes is stated, though the way in which the regulation occurs is still almost entirely unknown to us. All the same our understanding of this part of metabolism has deepened considerably, compared to what it was 50 years ago. We can now see clear-cut problems where formerly they were not even suspected, and to see a problem clearly before us is the first step towards its solution.

Here we must say something, albeit briefly, about the structure of proteins and of protoplasm of which they are so important a constituent. The amino acids which form the natural proteins are α amino acids ,which are linked so as to form long zigzag chains of polypeptides. This is at least the general assumption; according to PAULING [498] practically all proteins in the protoplasm are present as globulins, that is to say not in the form of long chains but folded. Because these chains are formed by residues of different amino acids, the R', R'' and R''' may vary considerably. Since more than 100 residues of amino acids can be derived from the natural proteins it is evident that they occur in an immense variety.

The constituents of protoplasm are proteins, fats and lipoids, constituting together about 25% of it, the remainder being water with ions in solution. The picture drawn by the biochemist FREY-WYSSLING [186] may give us some conception of their structure. Fats are esters of glycerin and fatty acids, and in the scheme shown below they are represented by a trident without a handle; the long carbon-chains of the fatty acids are the prongs and the residue of glycerol is the bar linking these together.

Lipoids have a more complex structure. Let us only consider lecithin. This may be regarded as a fat of which the residue of one of the fatty acids has been replaced by phosphoric acid, this in turn being esterized with choline $(CH_3)_3\,N\langle^{CH_2.CH_2.OH.}_{OH}$ The free acid group of the phosphoric acid will have a negative charge, the choline will have a positive charge which causes both groups to bind water. Thus

they are lyophilic, whereas the residues of fatty acids are lyophobic i.e. water repellent. For this reason the lecithin molecule is represented in the scheme by a tuning-fork, of which the residues of fatty acid form the two legs and the residue of phosphoric-acid-choline the handle pointing in the opposite direction, while the glycerol constitutes the bar linking them up.

In the case of protoplasm, the proteins with their long main chains may be taken to represent the meshes of the network of the gigantic micellae of the protoplasm, in which the various protein molecules are joined together at the so-called knots. This is illustrated by the scheme given here, which shows, moreover, that there may be various bridges. By ester-formation or by the formation of ethers, acid or amide, bridges may be set up, whilst with some amino acids, such as cysteine, a sulphur bridge of -S-S- arises as a result of reduction.

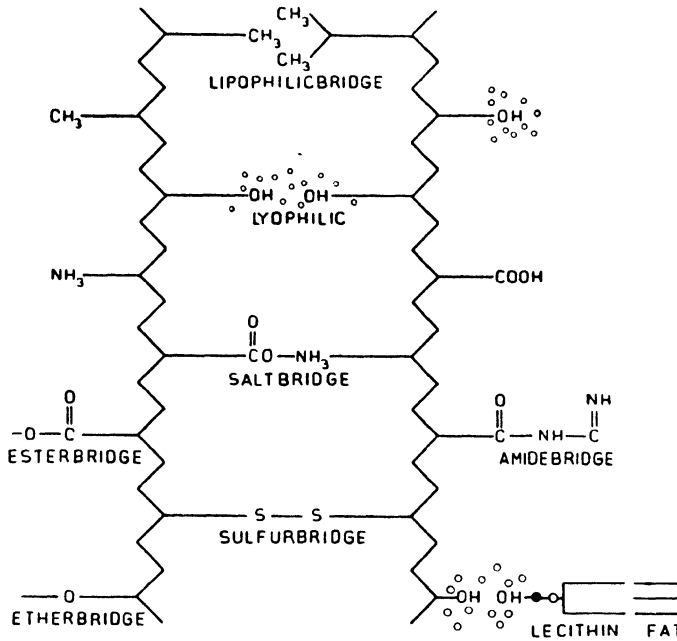


FIG. XVIII. Protein scheme of Frey-Wyssling

The formation of such a network must be visualized, not in one plane, but in space, the knots not necessarily remaining constant, but capable of being changed continually as and when the environment requires it. The formation of the lyophilic bridge is dependent upon presence of water molecules; that of an ester bridge upon the degree of acidity, the p_H, of the environment; that of a sulphur bridge upon the degree of oxidation, the so-called oxidation-reduction or redoxpotential. The lyophobic or lipophilic bridges, which may arise in the presence

of a -CH_3 group, are sensitive to temperature, for they become detached when the temperature rises above a certain value.

In colloid-chemical terms, the protoplasm is a polyphasic-emulsoid system and at the interfaces the curious reactions in interfaces occur, such as those of enzymes, of which our knowledge is still in its infancy.

It is clear that the living protoplasm is thus a dynamic whole, the condition of which is governed by factors of environment: by temperature, by the degree of acidity, by the oxidation-reduction potential or the presence of ions. There are continual changes in its condition in accordance with the changes in the environment. This is one of the most characteristic properties of living substances; the setting in of a stationary condition is a characteristic of the death of the protoplasm.

(J) Metabolism of Storage Substances and other Products.

Although fifty years ago the chief storage products and their localisation were fairly well known, the way in which they were formed and utilized again remained obscure. Of starch alone a little more knowledge had been attained, but even of this substance much remained obscure, because its chemical structure was undefined.

Out of the innumerable investigations made in regard to metabolism during the past 50 years it is possible only to mention a few in order to give an idea of the formation and breakdown of the substances concerned. We will deal first with the non-nitrogenous, next with the nitrogenous storage products, and finally with other important products of metabolism.

STARCH. The chemical composition of starch remained unknown for a long time, and even now this problem has not been solved entirely. The results of the work of H. P. WIJSMAN [747] have become the basis on which our knowledge of both the structure of the starch molecule and the nature of the various enzymes which cause the breakdown of starch, has been built up; this was clearly shown by G. A. VAN KLINKENBERG [330].

After much controversy the conception of one very large molecule has come to be generally accepted, in contradistinction to the old polymerization theory of P. KARRER, [310] according to which starch was supposed to be composed of only a few residues of glucose. This large molecule is assumed to be composed of a long chain of glucose molecules, linked together by principal valences. According to the position of these links a distinction is made between α and β starch. This has a bearing on the fact that the enzyme, formerly termed diastase, consists of two enzymes, already distinguished by WIJSMAN and nowadays indicated by the terms of α and β amylase. By their action the α starch is broken down into maltose, the β-starch into maltose and α amylodextrin. α Starch consists of non-bifurcate chains of glucose residues, β starch of bifurcate ones.

Morphological formation of the starch granule from crystal needles that are radially arranged, a hypothesis first put forward by ARTHUR MEYER [425], is supported by the work of AMBRONN [6] with the polarisation microscope and also by X-ray research carried out by J. R. KATZ [314].

During the past few years it has become more and more probable that the synthesis of starch occurs at the expense of glucose and with the aid of the enzyme phosphorylase. As a result of the work of C. S. HANES [229] it has even proved possible to obtain a product in vitro, which assumes a blue color when brought into contact with iodine. But this product does not possess the morphological structure of the starch granule, obtained through the action of the amyloplast, and the chemical structure is probably also slightly different.

As already mentioned above the relation of sucrose to starch has also been elucidated by HANES. He found that phosphorylases catalyse the reversible actions of starch $\rightleftarrows$ glucose-1- phosphate and of glucose-1-phosphate $\rightleftarrows$ sucrose.

The fact that in normal metabolism the synthesis of starch in the cell occurs at the expense of monoses does not prevent other substances such as mannose, galactose, sucrose, and even mannitol and glycerol from being able to play a part in this formation, as was shown by the investigations carried out long ago by J. A. BOEHM [55] and since confirmed by numerous other investigators.

The conversion of sugars into starch and vice versa repeatedly formed a subject of study: I need only mention the work of BROWN [80] and MORRIS [440] S. RYWOSCH [563], H. SCHROEDER [589], T. HORN [275], D. TOLLENAAR [655] and B. J. D. MEEUSE [418a].

By placing parts of leaves in solutions of sugar of varying concentrations it was found possible to cause formation of starch even in plants where this does not normally occur unless it be in the stomata. It is also possible to bring about a breakdown of starch in the tissues by too high a concentration of sugar in the solution. During the wilting of leaves the quantity of starch decreases, whereas that of sucrose increases. Evidently the osmotic value plays an important part in this respect. In Chapter III the formation of starch in the cells at the expense of a solution of glucose or sucrose has been discussed from the viewpoint of permeability or non-permeability.

Likewise interesting were the investigations concerning the conversion of starch into sugar and vice versa in stomatal cells, a subject already mentioned in the discussion on transpiration. The concentration of ions was then found to be of importance in this connection; this may be regarded as an indication that these conversions are less simple than they appear.

INULIN and GLYCOGEN. Here I will not go into further detail regarding the conversion of glycogen in Fungi. The function as reserve carbohydrate has never been doubted and little research has been done in this

respect. Into the subject of inulin I must go somewhat deeper. From the tubers of Compositae, such as *Dahlia variabilis* and *Helianthus tuberosus* an enzyme was obtained, termed inulase, which converts inulin into fructose. These tubers contain large quantities of inulin, sometimes up to 50% of the dry weight, which quantity decreases considerably when the new buds are sprouting, so there is little doubt of its function as a reserve substance. This was confirmed by GRAFE [209] and VOUK [686] who also proved that inulin is formed in photosynthesis and translocated to the tubers.

GLUCOSIDES. The function of glucosides as reserve substances, though plausible in connection with the carbohydrate in the molecule, has, however, been doubted somewhat. In many cases this doubt is justified. Though there are glucosides whose function in this capacity has been proved experimentally.

Glucosides are substances which are broken down by a glucosidase, formerly termed emulsin, and besides glucose some aglucon or other is formed in the process. This aglucon may be an aromatic product, for instance hydroquinone is formed by the breakdown of the glucoside arbutin ;or it may be a nitrogenous product which yields hydrocyanic acid, such as occurs in the hydrolysis of amygdalin.

The present writer was able to demonstrate the storage function of various glucosides, e.g. of arbutin and salicin. N. J. STEKELENBURG [624] did the same for various hydrocyanic acid glucosides.

The bast of some species of willows, particularly that of *Salix purpurea*, contains the glucoside salicin which is hydrolysed into glucose and saligenin, ortho-oxybenzyl alcohol, by a glucosidase that is present in the plant. A comparison of the quantities of various storage substances in wood and bast before and after the sprouting of the young shoots shows a clearly discernible decrease of all storage substances. During the sprouting of cut twigs put in water both in the dark and in the light, the relative decrease of salicin is considerably greater than that of carbohydrates or proteins. Also in the leaves this glucoside salicin functions as a storage substance. During the day, when there is photosynthesis, the salicin content increases, but it decreases again during the night as a result of enzymatic breakdown, the glucose being translocated to the stem. It is curious that the aglucon saligenin undergoes rapid quantitative conversion into the diphenol, catechol or pyrocatechin, which later, when glucose is again present, is utilized for the formation of fresh salicin.

Among the investigations concerning hydrocyanic glucosides the experiments with *Phaseolus lunatus* are specially noteworthy. This species contains the glucoside phaseolunatin which is broken down into glucose, hydrocyanic acid and acetone. When leaves are cut off and placed in water in the dark, the phaseolunatin decreases; by the addition of glucose to the water the glucoside is protected against this decrease. This fact clearly indicates its function as a carbohydrate

reserve. Also after adding asparagin to the solution of sugar, STEKELENBURG observed an increase of the glucoside in the leaves. This definitely does not support TREUB's [657] theory that hydrocyanic acid is the primary assimilation product of nitrogen; rather is it to be regarded as a by-product of protein synthesis.

Of other glucosides, such as the anthocyanins, the red pigments in the flowers, leaves and fruits, it can hardly be assumed that they have any reserve function. K. NOACK [462] formulated the theory that there was an equilibrium in the plant between the aglucons of the anthocyanin and the flavones, complex yellow organic pigments, the position of the equilibrium being dependent upon the oxygen potential, i.e. upon the rate at which oxygen is liberated in photosynthesis and utilized in respiration. Experimental research by L. W. KUILMAN [355] in the writer's laboratory did not confirm this theory. Though in buckwheat seedlings KUILMAN did find that the formation of anthocyanin results from combination of a purely chemical process with a photochemical process and is greatly assisted by the occurrence of warm, well-illuminated periods alternating with cold and dark ones.

FATS. These substances are glycerides of various fatty acids, saturated as well as non-saturated, which may be present in the protoplasm of different cells, both in the higher and the lower plants. According to the investigations carried out years ago by C. W. NAEGELI [449], in the great majority of higher plants fats constitute the non-nitrogenous reserve substances of seeds. In the past century it was known, furthermore, that such oleiferous seeds have abnormally low respiratory quotients during germination. Whereas in seeds containing starch as the non-nitrogenous storage substance this quotient amounts to about 1, it is in oleiferous seeds, sometimes no more than 0,3. This fact which was observed by LECLERE DU SABLON [368] in *Ricinus communis*, led to the view that during germination fat is partly dissimilated, partly converted into carbohydrates and that this conversion requires the binding of oxygen. SACHS succeeded in establishing such formation of starch in germinating oleiferous seeds.

The formation of fats in ripening seeds was also connected with conversions of carbohydrates, the high respiration quotient during that period being regarded as an indication of such conversion. PFEFFER observed such formation of fat at the expense of carbohydrates in unripe peony seeds which had been isolated from the motherplant.

But all this did not answer the question how those conversions occur: for this a greater knowledge of the chemistry of metabolic processes was required. The work of S. L. IWANOW [290] and others gradually helped to make it clear that in germination first fatty acids arise from the fats and in the formation of a fat the fatty acid is also produced first. The question is, therefore, how does the fatty acid originate? As will be seen presently, there are good reasons for associating the formation of fats with processes of dissimilation, i.e. breakdown of

glucose. From what has been said about respiration, this will at once be clear as far as glycerol is concerned, and we shall see that the same argument applies in the case of fatty acids.

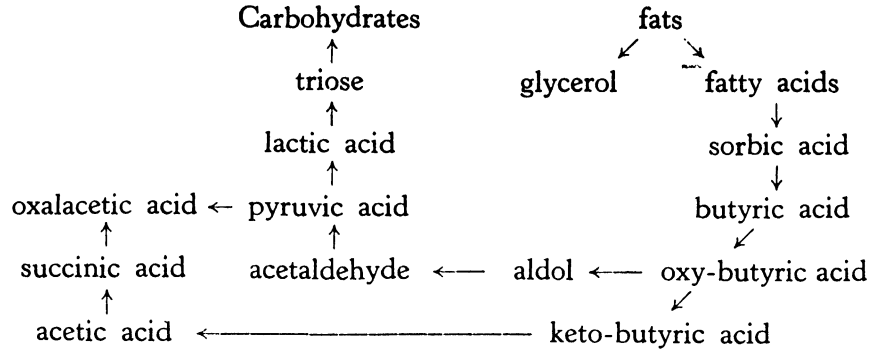
H. EULER [163] already pointed out the significance of the process of aldol formation from the acetaldehyde produced in respiration. In the beginning of this century he argued that by the splitting off of water this aldol might yield crotonic-aldehyde, which as a result of reduction is converted into butyraldehyde. In this way butyric acid may arise in the metabolism of bacteria which are species of the genus *Clostridium*. As a result of continued actions of an oxido- reductive nature, the higher fatty acids may also originate.

A study of the fat formation in a kind of yeast, *Endomyces vernalis* in 1925 led H. HAEHN [223] and W. KINTTOF [326] to formulate a similar hypothesis, by which it was assumed that the synthesis was brought about with the aid of the products of dissimilatory metabolism by virtue of a series of voluntary reactions.

In the higher plants the breakdown of fats during germination of the seeds has also been studied in this manner, but not the synthesis. The most important investigation is that of ZELLER [752] and ENSER [159], who studied the germination of the oleaginous seeds of *Cucurbita* and *Helianthus annuus*. They found that a dehydrogenase is present in these seeds, which dehydrogenates the saturated fatty acids into non-saturated ones, in this case into oleic acid. Investigations by S. ISAAC [287] and R. SIEGEL [604] concerning the breakdown of animal fats led ZELLER and ENSER to assume that sorbic acid, with the formula CH_3 -CH = CH — CH = CHCOOH, was the intermediate product. This acid was in the higher plants known only in the rowan tree or mountain ash, *Sorbus aucuparia* and the assumption of the authors was that in the metabolism of fats its breakdown took place so rapidly that its presence had so far escaped notice.

In their investigations concerning the germination of oleaginous seeds ZELLER and ENSER applied the methods used by NEUBERG [456] for the study of respiration and described in Chapter II. They injected sorbic acid into the tissues of *Cucurbita* seeds and found that at a particular stage of germination the formation of starch increased by about 20% and that sucrose was also present.

If potassium sorbate is added to an autolysate of sunflower-seedlings, the starch will increase instead of decrease, which does show the importance of this substance in the intermediary fat metabolism. Pyruvic acid, oxalacetic acid, acetaldehyde and β keto-butyric acid also cause starch to increase instead of decrease, whilst β oxy-butyric acid, lactic and succinic acid tend to inhibit the breakdown. These facts led ZELLER and ENSER to draw up the following scheme for the conversion of fats into carbohydrates:



This of course, provides no proof that the conversion does occur in this way, though it cannot be denied that ZELLER's views seem plausible enough; there is, at least, circumstantial evidence and in any case they serve to point the way for further research.

PROTEINS. Among the nitrogenous reserve substances proteins are by far the most important. Those contained in seeds have been studied most closely. The work of TH. B. OSBORNE [481] showed that globulins constitute their chief component, although albumins and sometimes prolamins (proteins which are soluble in alcohol) are present in cereals. As was stated above, it was established experimentally in the course of the present century that proteins are formed out of a series of linked amino acids, though it would lead us too far into chemical territory to go into details. Experiments of VICKERY [679] c.s. with the isotope N. 15 provide evidence that continuous chemical interaction occurs between the nitrogen of tissue constituents and of supplied nutrients.

Research concerning the protein metabolism in plants was hampered by so many difficulties that at first it confined itself more or less to the study of what happened during germination of a few kinds of seeds. It was soon found that there is no question of a translocation of proteins as such and that breakdown into amino acids plays an important part. But the products of the breakdown of proteins in vitro are not encountered in the seedling. Instead of a score of different amino acids, observed in vitro, only a few substances arise in the breakdown of proteins in the living seedling, viz. the acid amide of aspartic acid, asparagine and its homologue the acid amide of glutaric acid, glutamine, BOUSSINGAULT [67] discovered this before 1870, but it was especially PFEFFER, who went into the question more deeply. He found that growing points sometimes contain approximately 25% of asparagine calculated on a basis of dry weight, and that the accumulation of asparagine became noticeable especially in the absence of carbohydrates. Hence, etiolated seedlings of lupin, *Lupinus luteus*, a plant with seeds that are rich in protein, though poor in carbohydrates, proved the most suitable object for his investigations.

PFEFFER was of the opinion that there is a sharp contrast between the

breakdown of proteins in the living seedling and that in vitro. He considered that in the second case no asparagine was formed. E. SCHULZE [591] however, pointed out that the contrast was by no means so very great, for though in the hydrolytic breakdown in vitro asparagine does arise, it is broken down secondarily into ammonia and aspartic acid. Notwithstanding this, the difficulty remains that in the breakdown in vivo much more asparagine arises than in vitro and this substance is not broken down.

The obvious assumption is that in the living plant asparagine must be a secondary product, formed by virtue of synthesis from aspartic acid and ammonia, and this assumption is confirmed by the fact that in the absence of oxygen protein dissimilation in the plant will yield ammonia, but hardly any asparagine, whilst it is a fact that for syntheses the presence of oxygen is usually essential.

BUTKEWITSCH [101] observed in 1909 that narcosis of lupins germinating in the dark and in an atmosphere containing toluene-vapour will reveal the presence not of asparagine but of ammonia. This of necessity leads us to the research of the nestor of Russian plant physiologists, D. N. PRIANISCHNIKOW [518]. This investigator studied the germination of oat, *Avena sativa*, of lupin, of *Cucurbita* spec. and a few other plants. He caused 100 lupin seeds to germinate (a) in water, (b) in water plus ammonium sulphate $(NH_4)_2\ SO_4$, (c) in water plus ammonium sulphate and calcium carbonate. In solutions (b) and (c) the plants became ailing and the seedlings contained less asparagine and much more ammonia than the controls which were germinating in water. Compared with *Cucurbita*, *Lupinus luteus* contains far fewer non-nitrogenous reserve substances, and when glucose is added to lupin seedlings, they will behave like cucumber. Seedlings of *Avena sativa* already 3 weeks old which have used up their reserve of carbohydrates owing to germination in the dark, behave like lupin with its excess of nitrogenous reserve substances.

Clearly the main point is the ratio between nitrogenous and non-nitrogenous storage substances. If the latter are present in sufficient quantity, synthesis of asparagine occurs; if not, ammonia, the product of dissimilation or the breakdown of protein accumulates and damage occurs.

PRIANISCHNIKOW summarized this in the following scheme:

Conditions		Result	
carbohydrates	light	synthesis of asparagine	NH_3-accumulation
+	—	+	—
—	—	—	+
+	+	+	—
—	+	+	—

Hence, according to PRIANISCHNIKOW, asparagine is the substance in which the higher plant ties up the toxic substance ammonia, thereby rendering it harmless. Thus ammonia becomes the final step in protein dissimilation and at the same time the first in protein synthesis or, in PRIANISCHNIKOW's words, ammonia is the alpha and omega of protein metabolism.

In the instances mentioned above, asparagine or glutamine might be regarded as the second stage in the synthesis during germination but perhaps it would be more in keeping with the facts to consider them as mobile storage products. As was mentioned above when we we discussed the process of transamination the acid amides, asparagine or glutamine, are the form in which the aminoacids are safeguarded against transamination.

VICKERY [679] c.s. consider PRIANISCHNIKOW's view that synthesis of amides results from an effort of the cells to maintain a low level of ammonia to be inadequate to account for the behaviour of these amides. The amount of the latter substances is in VICKERY's opinion determined by the amount of nitrogen-free precursors as well as by the amount of ammonia present.

From the roots of germinating barly. *Hordeum sativum*, CHIBNALL [110] and GROVER [215] isolated an enzyme that splits off ammonia from asparagine, which might lead to the assumption that under special conditions this enzyme may also be capable of effecting the synthesis of of asparagine, S. KOSTYTSCHEW [344] suggested that oxalacetic acid might play a part in the formation of asparagin.

$$\mathrm{COOH.CH._2CO.COOH + 2\,NH_3 \rightleftarrows}$$
$$\mathrm{+(NH_2)\,CO.CH_2.CH(NH_2).COOH + H_2O + O}$$

For our inderstanding of the connection of the entire metabolism it is rather interesting to encounter again a substance like oxalacetic acid, which may be concerned in the carbohydrate metabolism. This calls to mind the investigations of STEWARD [629] and PRESTON [517] which we discussed in connection with protein synthesis. Here we must also refer to the transamination studied by BRAUNSTEIN [72] and KRITZMANN [350], which agrees with KOSTYTSCHEW's suggestions. These investigators found that the reaction between pyruvic acid, on the one hand and aspartic acid or glutaric acid on the other, is the one that occurs most rapidly.

CHIBNALL [110] suggests that the citric acid cycle of KREBS [348] and JOHNSON [301] provides a chemical mechanism whereby both oxalacetic acid and α ketoglutaric acid may be formed. The acids may react with ammonia to form aspartic and glutaric acid in the presence of the special enzymes and react further with ammonia to produce amide.

Recent work by WOOD [741], EVANS [164] and SLOTIN [610] using isotopic carbon has suggested that cis-aconitic acid rather than citric acid is an intermediary between oxalacetic acid and α keto-glutaric acid,

citric acid being formed as a side reaction. As was pointed out by VICKERY [679], perhaps the strongest argument in favour of these schemes is for the present that they permit a rational explanation of relationships between well known plant constituents and respiration.

To mention all these different investigations in detail would exceed my limits. I will quote the conclusion of WOOD, and CRUICKSHANK [126] that when leaves are starved in air, protein decrease is closely correlated with the decrease in amount of soluble carbohydrates. When leaves are starved in nitrogen loss of carbohydrates occurs as rapidly as in air; respiration, however, falls to a low level and protein content decreases only very slightly except in case of injury. In nitrogen proteolytic enzymes may be prevented from coming in contact with their substrate, perhaps steric hindrance might retard protein hydrolysis.

As we pointed out in the discussion above protein synthesis and dissimilation always occur simultaneously and the same is, of course, also true as regards the metabolism of amino acids. Research carried out with the aid of the N^{15} isotope has provided conclusive evidence of this in respect of animals, as well as of plants. For this reason it is worth while to select conditions in such a way that one of the two processes can be practically neglected. This is still only possible with regard to synthesis.

Investigations of this kind concerning the breakdown of amino acids have been made by H. P. J. M. VAN WAESBERGHE [691] and by A. GORTER [205]. The object used was in both cases *Aspergillus niger*. Exposed layers of mould were rid of their reserve substances by leaving them standing on water for a while. Such starved layers of mould, in which the so-called basal metabolism prevails, disaminate amino acids, and it was found that such disamination occurs under uptake of oxygen. The course of events was studied more closely in asparagine where the oxidative disamination was found to be a complicated chemical process closely associated with the respiration of the cell and most likely taking place with the aid of non-specified enzymes. Once again we are struck by the relation between nitrogen metabolism and respiration.

UREA. As compared with proteins, all other nitrogenous storage substances take very much a secondary place. Small quantities of urea are found in many plants as is shown by the work of G. KLEIN [328] and K. TAUBÖCK [645]. Only in a few Fungi, e.g. Gasteromycetes, such as *Lycoperdon* was N. N. IWANOW [289] able to demonstrate the presence of larger quantities, and it is only in those instances that urea can be regarded as a storage product.

ALKALOIDS. Formerly, alkaloids were regarded as waste products, substances that once formed, did not re-enter the metabolism. But this is not correct in all cases, for not only are some alkaloids again assimilated in the metabolism in the event of nitrogen deficiency, but in normal cases also this may occur regularly. This becomes most

evident with the xanthine derivatives, caffeine and theobromine, which are present only in a few genera belonging to entirely different families, viz. the genera, *Coffea, Thea, Cola, Paullinia* and *Theobroma*. In the genus *Ilex* only a few species contain alkaloids and in the former genera there are sometimes species in which they are lacking. *Coffea Thea* and *Ilex* contain caffeine. *Cola, Paullinia* and *Theobroma* theobromine and caffeine.

The formation of these alkaloids, which the present writer studied in Buitenzorg (Java) and in Brazil, occurs during the formation of proteins in the growing points and young leaves, as was explained above. In the young leaves the quantities of caffeine and theobromine first increase, but sooner or later, usually before the leaves have reached their ultimate size these quantities begin to decrease. In several instances the present writer succeeded in demonstrating that there is in these cases no question of a translocation as such, nor loss as a volatile nitrogen compound, but that these xanthine derivatives provide material for fresh protein synthesis.

The character of the xanthine derivatives as storage substances becomes still more evident in a study of germination. Seeds of *Paullinia Cupana* have a fairly high content, up to a maximum of 5% calculated on a basis of dry weight. During germination the xanthine derivatives are broken down and utilized again for new protein synthesis as was proved by experiments with *Coffea* and *Theobroma*. Analysis of the whole of the seedling does not, as a rule, render this evident, because the decrease referred to is offset by the formation of new alkaloids in the young shoot. The importance of the alkaloids to protein synthesis is illustrated by the following argument. When comparing two species of plants, the seeds of which are very similar in size, protein content and also in the content of non-nitrogenous reserve substances, one species containing caffeine or theobromine while the other does not, the decrease in protein during germination will be found to be much greater in the species without xanthine derivatives, whilst the proportional change in the dry weight will be about equal. This is in keeping with the fact that seeds containing xanthine derivatives have a low protein content. This evidence, if not altogether conclusive is assuredly circumstantial. The present writer likewise found a connection with protein metabolism in species with other alkaloids. J. C. J. WALLEBROEK [696] made a study of *Lupinus luteus* in this respect.

ORGANIC ACIDS. There is, perhaps, no other detail of plant physiology, the historical discussion of which gives us such a clear picture of the way in which this science has changed its approach and methods of research in the course of time.

In 1888 HUGO DE VRIES carried out physiological investigations concerning the Crassulaceae and found that organic acids increase during the night. He furthermore found that these substances decreased the following morning and that this decrease occurred more rapidly

in light than in the dark. In connection with his research concerning the turgor of the cell, he laid particular stress on the importance of organic acids, and especially of their potassium salts, for the osmotic value of the cell.

At about the same time AD. MAYER [417], G. KRAUS [346], O. WARBURG [698] and E. AUBERT [21] investigated the exchange of gases in the succulents. At night these plants take up oxygen, but produce hardly any carbon dioxide. They associated the formation of the acids during the night with a supposed oxygen deficiency in the intercellular spaces of succulents such as Crassulaceae. The production of carbon dioxide during the following day and particularly under illumination they assumed to be advantageous to the plant in that this carbon dioxide could be utilized at once and did not get lost in exhalation. They argued that hence the exchange of gases could be less intensive, an important advantage to such succulents in the struggle for existence. The stomata could be closed during a great deal of the day and the transpiration less intensive.

Thus in both cases the argument was on purely teleological lines: the plant forms its organic acids because these are needed for the turgor of the cells; the plant — by intermediate production of organic acids — delays its formation of carbon dioxide, so that this may be utilized in photosynthesis, whilst at the same time transpiration can be reduced. The argument that the assumed low oxygen tension in the tissue caused production of the organic acids was not very plausible.

Likewise entirely teleological was the view of E. STAHL [620], who held that oxalic acid was formed in different plants so that in its calcium salt a substance should be produced which in the form of raphides would serve as a protection against snails. To put it mildly, this sounds most peculiar to the modern scientist, but the biologist of the last few decades of the past century was led to these views by his constant concentration on the problems of natural selection and the struggle for existence. Experiments have since completely overthrown the protection theory, at least in this case.

During the present century views arose which associated the formation of acids with the entire metabolism. In 1903 W. BENECKE [38] demonstrated that the oxalic acid formed was fixed as calcium oxalate with the aid of calcium ions, though this did not explain the formation of the acid itself. He did find that a species which usually produced free acid, was able to produce the salts from this or leave them unproduced, according to circumstances. In this regard the source of the nitrogen was found to exert some influence, for when this element was provided in ammonium compounds instead of in nitrates, plants which usually formed calcium oxalate did not produce this salt, or produced it to a lesser extent, even if the calcium supply was not varied. This was particularly noticeable in those species which produce tetragonal calcium oxalates, but less in those which naturally form raphides. This different formation is in connection with the properties of the

solution in the vacuole. BENECKE, moreover, established that some species were able to convert the oxalic acid first formed into carbon dioxide.

In those days acid formation was generally regarded as part of carbohydrate dissimilation, the acid being assumed to be a kind of incomplete oxidation product of a carbohydrate. To some extent this view was based on the experiments of C. WEHMER [705] with *Aspergillus niger*, species of the Ascomycetes, which has so frequently been the subject of physiological research. In connection with this particular subject, the Russian physiologist S. KOSTYTSCHEW [344] suggested that nitrogen metabolism might also play a part in the formation of organic acids. This idea especially seemed an attractive one in respect of the formation of citric acid with its branched carbon chain. In agreement with F. E. V. ELFVING [155], KOSTYTSCHEW expressed the view that isoleucine one of the products of the breakdown of protein by disamination and oxidation would yield citric acid.

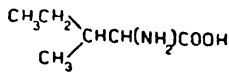


FIG. XIX. Isoleucine

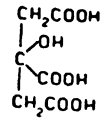


FIG. XX. Citric acid

With regard to the higher plants this idea was further elaborated by W. RUHLAND [560] and his pupils. RUHLAND and K. WETZEL [715] first ascertained the metabolism of *Begonia semperflorens* in general, with a view to acid formation. In its metabolism ammonia was found to play a more important part than in the normal nitrogen metabolism. For instance, in the morning the ratio of ammonia nitrogen to amino acid nitrogen is 1 : 2, whereas in other plants that ratio is 1 : 50. If the authors placed the *Begonia* in the dark, there would be strong protein dissimilation at 35°C, so that after a few days 30% of all the nitrogen was present in the form of ammonia nitrogen. RUHLAND and WETZEL associated this with the experiments on *Aspergillus*, which in a peptone environment converts 60% of the peptone into ammonia and simultaneously binds this to oxalic acid. According to them in *Begonia* there must also be some connection between such accumulation of ammonia and the formation of organic acids. If less ammonia is formed, the amino acids accumulate, hence the idea that organic acids may originate from disaminated amino acids seems a plausible one. It is supported by the fact that as soon as the carbohydrates of *Begonia* have been used up as a result of the plant being placed in the dark, the production of oxalic acid begins simultaneously with the breakdown of amino acids, although up to that time the production of oxalic acid has been insignificant.

In young leaves, not removed from the plant, intensive decomposition of amino acids occurs during the night coupled with the formation of ammonia. Yet the total quantity of nitrogen compounds and amino acids of those young leaves increases, from which it may be concluded that nitrogen is translocated to them in the form of amino acids. If there is hydrolysis of the proteins in old leaves or parts of the stem, the amino acids go to the young parts and are broken down there under formation of oxalic acid and ammonia, the last being used again for protein synthesis after a temporary binding to acid. Thus we are again reminded of PRIANISCHNIKOW's statement that ammonia is the alpha and omega of protein metabolism.

With the rhubarb plant, *Rheum Rhabarbarum*, RUHLAND and WETZEL came to the same conclusion, though this cannot be entered into, as T. A. BENNET CLARK [39] and W. M. WOODRUFF [743] were unable to confirm these results. The latter are inclined to associate the formation of acids with the metabolism of carbon, thus reverting to the old idea. Earlier, J. WOLF [738] came to a similar conclusion as a result of his study of the acid formation in the Crassulaceae. Recently VICKERY and co-workers have investigated RHEUM in detail.

In contradistinction to *Begonia*, there is in the Crassulaceae no question of an abnormally strong formation of ammonia. The conversions occurring in the protein metabolism are, in fact, too slight for a direct relation between the breakdown of protein and the formation of acid to be possible in this instance. This particular objection may also be raised in respect of the metabolism of *Begonia*.

The connection between organic acids, in this case chiefly malic acid, and the oxidative breakdown of sugar is shown by the fact that oxygen is essential to the formation of malic acid; the absence of oxygen inhibits the formation of acid to the same extent as does the presence of hydrocyanic acid or narcotics. There is also a relation between the quantity of sugar that has disappeared and the quantity of malic acid produced. WOLF assumed that the breakdown of sugar — in the Crassulaceae a special sugar with 7 C atoms, sedoheptose — occurs in an unusual way. BENNET CLARK shares this view, though he differs on points of detail. This is not surprising, for the carbohydrate dissimilation of most of the higher plants is still virtually "terra incognita". To some extent the enzymes that are active are known, but it is not yet possible to visualize the entire process. BENNET CLARK's hypothesis that the process involves a resynthesis of carbohydrates from malic acid, an assumption which brings to mind BLACKMAN's [52] observations and conclusions concerning the respiration of ripening apples, may be correct, but is not yet proved.

Hardly anything has been said, so far, concerning the huge number of substances, both aromatic and non-aromatic, which are not present generally but only in a few genera of plants. In most cases their physiological significance is unknown; it is even doubtful whether they

have any particular function in metabolism or in any other respect.

A short while ago the present writer pointed out that every genus of plants, and sometimes even every species of one genus, is characterized by a definite combination of substances, such combination being as much a chemical characteristic of the particular genus or species as the combination of morphological and anatomical pecularities. No particular biological significance need be associated with either of these combinations, however.

Chapter VI

TRANSLOCATION OF ORGANIC SUBSTANCES

(A) Introduction; Research before 1895.

A review of the state of botanical science at the end of the past century brings out how widely held was the opinion that the organic substances, produced in the leaves, were translocated by certain tissues and along special routes to growing points and to storage organs. To quote Hugo de Vries: "the route for proteinaceous substances lies in the phloem, particularly in the cribriform ducts, while glucose finds its path in the parenchyma by way of cylindricals cells, the starch-sheath". In support of this assumption were cited the ringing experiments carried out with branches on the plant as well as with cut branches which formed roots. It was said that experiments with Dicotyledonous plants showed that the marrow does not constitute a transport route: Dicotyledonous plants with bicollateral bundles making it evident that this route lay especially in the phloem, since transport could continue in these when a ringing wound was made, whilst in plants where bicollateral bundles are lacking transport is stopped by ringing.

Critical consideration of this view will show, firstly that no strict proof is given for the assumption of a transport of protein along the phloem side by side with a glucose translocation along the parenchyma, secondly that no mention is made of the way in which such transport is brought about. Yet this second point did occupy de Vries's attention. The fact that diffusion over long distances is extremely slow and does not provide a satisfactory explanation of the transport led de Vries to formulate the hypothesis that some part might be played by protoplasmic streaming. He suggested that this might cause an acceleration of transport in each cell individual to such an extent as to leave only the difficulty of the diffusion from cell to cell. But de Vries submitted no proof that such protoplasmic streaming did, in fact, occur in the transport routes, and this may be the reason why the hypothesis is not mentioned in his Textbook. At his Capita College of about 1900 de Vries cited the publication of J. H. E. F. Kienitz-Gerloff [323], in which this view was put forward, with obvious approval.

It appears, therefore, that DE VRIES was in complete agreement with the view of SACHS and also based his opinions on VON HANSTEIN's [233] experiments with ringed branches of Dicotyledonous plants with collateral and bicollateral bundles.

The view that proteins and sugars were translocated along different routes, the former through the phloem and the latter through the parenchyma, along the starch-sheath, had been given prominence by SACHS in particular. This investigator overlooked, however, that TH. HARTIG [239], as early as 1859, observed a high sucrose content in the juice from the sieve tubes. In 1894 G. KRAUS [347] found that such juice taken from *Cucurbita* had a dry weight of 10%, comprised of 20% proteins, 40% amino acids and 38% carbohydrates (sucrose).

The theory of carbohydrate translocation by way of the parenchyma entails some rather awkward assumptions. The translocation from cell to cell along the starch-sheath must take place in solution, so that in each cell fresh starch must be formed from this, only to be broken down again in order to pass on to the next cell. The accumulation in the form of starch is of no help in translocation; indeed, it may be said that the very presence of starch is an argument against a carbohydrate transport in solution.

In fact, as early as 1888 H. HEINE [245] had shown by means of ringing experiments on the stems of *Phaseolus* seedlings that the starch in the starch-sheath had no connection with carbohydrate transport. Hence VON HANSTEIN's conclusion that the translocation of organic substances passes along the sieve tubes is the most logical one, but its mechanism remains unexplained. STRASBURGER [636], in his painstaking observations, failed to find any protoplasmic streaming in this part and, moreover, diffusion over long distance is far too slow, as was stated above.

(B) Investigations and Theories since 1895.

Reviewing the various publications since 1895, we find that an attempt to throw fresh light on this question of translocation was made in 1897 by F. CZAPEK [130], one of PFEFFER's pupils. He asked two questions, viz. (1) whether living elements play any part in the translocation, and (2) whether this process takes places in a straight line. CZAPEK tried to find an answer to the former question by plasmolysing or narcotizing the bast of the branches, and found that translocation continued after plasmolysis, though not after narcosis. If his experiments were reliable, the living protoplasm might undoubtedly be regarded as playing a part in the process, but unfortunately they could not stand up to criticism. DELEANO [135] justly regarded the experiments concerning plasmolysis as being of little value, because it was by no means certain that plasmolysis did, in fact, occur. In his test relating to narcosis, CZAPEK paid attention only to starch and not to the soluble carbohydrates. When DELEANO studied the subject, he found that along

the narcotized leaf-stalks these products were being carried away to half the normal extent; hence he concluded that translocation is possible even in a state of narcosis.

In regard to the question whether translocation occurs in a straight line CZAPEK made ringing experiments in which a bridge-connection in the form of a hook was maintained. He based his conclusions upon the place where there was most root-formation, but DELEANO justly pointed out that CZAPEK did not pay any heed to the polarity of the root-formation. If this phenomenon is taken into account, CZAPEK's tests are by no means conclusive.

Interesting observations were presented in 1909 by O. SCHNEIDER-ORELLI [581], who reported that starch would disappear from leaves when the xylem of the vascular bundles was destroyed as a result of the tunnelling action of mining caterpillars, but did not do so when the sieve tubes were destroyed. This seems to indicate that in the second case translocation is no longer possible, but again there is the objection that the soluble carbohydrates are not taken into account by SCHNEIDER-ORELLI.

Thus matters stood when about 1920 the problem began to be reconsidered by various investigators. Following HARTIG's lead, SACHS had declared that in the spring the state of affairs was exceptional in that in this season translocation of carbohydrates occurred along the wood. O. F. CURTIS [128] repeated the ringing experiments with sprouting branches and concluded that the wound obstructs the transport of organic substances in the apical direction. According to him, these substances did not disappear from the bast in a part between two ring wounds. CURTIS's experiments were not quantitative, but later GRETA SANDERS [567] made a similar investigation with quantitative methods in the present writer's laboratory and was able to confirm CURTIS's results, though this did not prove his conclusion that under those circumstances there is no translocation of organic substances along the wood. It only showed that then substances from the bast are not translocated along the wood, but proved nothing concerning the transport of storage products from the wood-parenchyma.

By centrifuging, H. H. DIXON [139] and W. G. R. ATKINS [20] obtained a reducing solution from the wood, in keeping with the long known fact that the bleeding-sap of various trees contains soluble carbohydrates.

Diametrically opposed to CURTIS, who denied all translocation of organic substances along the wood, was the view of LUISE BIRCH-HIRSCHFELD [50], also published in 1920. In the last publication to come from PFEFFER's laboratory, Mrs. Birch-Hirschfeld denied that translocation occurred along the bast. She based her views on the data presented by BROWN [80] and MORRIS [440] concerning the removal of material from the leaves of *Tropaeolum majus* and concluded that transport by means of diffusion would be 100 times too slow to be able to serve as an explanation of the results obtained. Somewhat diffidently

she suggested there might possibly be a downward translocation of organic substances along the wood.

In 1923 DIXON ventured to suggest this hypothesis, supported in the first place by the centrifuging experiments referred. Secondly DIXON emphasized the impossibility of a sufficiently rapid transport by diffusion. Using as object of investigation a potato tuber which obtained its nutriment through a runner of the motherplant, he proceeded to determine the total diameter of the phloem and the quantity of carbohydrates accumulated in the tuber after a certain length of time. Even assuming that there is a high sugar concentration in the sieve tubes, the rapidity with which the solution would have to move in these ducts was found to be so high as to be many times greater than the rate of diffusion. Hence DIXON concluded that translocation of organic substances takes place along the youngest vessels of the wood, those adjoining the cambium. He considered that the results obtained in VON HANSTEIN'S [233] ringing experiments were due to the cambium having become damaged when the wound was made.

In the present writer's opinion, DIXON'S conclusion was somewhat hasty and further proof from experiments was desirable. VON HANSTEIN'S ringing experiments on plants with bicollateral bundles constitute a strong argument against DIXON'S reasoning, because there would have been damage to the cambium and a stopping of translocation as much as in the case of plants with collateral bundles, and VON HANSTEIN'S experiments showed a continuance of transport in the plants with bicollateral bundles.

For this reason VON HANSTEIN'S experiments were repeated by the present writer, with the precaution of covering the wounds immediately with melted cocoa-butter (temperature 35°C) so as to avoid all possible damage to the peripheral wood. The results, however, remained the same and the contrast between *Nerium Oleander* with bicollateral, and *Salix purpurea* with collateral bundles was just as sharp, hence the transport of organic substances in *Oleander* cannot have been interrupted, though it must have been interrupted in the willow.

Ringing experiments on variegated branches of *Aesculus Hippocastanum* yielded similar results. If these are ringed before sprouting, the buds still sprout and the branches and leaves continue to grow for some time but because lack of chlorophyll renders photosynthesis impossible, they die after a few weeks with symptoms indicating water deficiency. Thus it seems that in this instance there is hardly any transport of organic nutrients along the wood: the closer the wound is below the bud, the sooner the young shoot dies off, hence the nutrient must be translocated along the bast, as stated by CURTIS. Water deficiency in the young shoot will result because the tissue lacks sufficient suction pressure and is not able to compete with other parts of the plant. If such experiments are made with green Oleanders, which sprout in the dark, so that photosynthesis in the young shoot is likewise

impossible, their branches will develop normally, due to the supply along the intraxylary phloem.

Hence these tests confirm the earlier view that translocation of organic substances takes place along the sieve tubes. The thesis of one of WINKLER's [734] pupils, EMMA KASTENS [311], published in 1924, was more or less in the nature of a compromise, for this author was of the opinion that the xylem also plays a part in the transport of organic nutrient substances, whilst the sieve tubes would serve for that of hormones which, as will be seen later, are required for sprouting. With this view she correlated the work of HABERLANDT [222], which will be discussed later. In isolated pieces of potato tissue HABERLANDT observed cell-division only when there was phloem in the tissue. Nowadays we should, perhaps, think of an auxin transport in the bast.

Investigations made by CH. COSTER [122] in my laboratory failed to confirm EMMA KASTEN's view. If COSTER loosened the bast from the wood of branches 3 and 4 years old, except at the apical side, he noted a clearly discernible increase in dry weight in these long strips during the summer months. Hence a transport of nutrient substances must have taken place in that strip of bast, as there was no possibility of independent photosynthesis.

The well-known fact that the sucking roots of a complete parasite like *Cuscuta europaea* are connected with both the sieve tubes and the wood vessels of its host, is an additional indication of the significance of the former in nutrition.

About 1930 two series of investigations were published, which, though starting from totally different points of view and being carried out with different methods, arrived at precisely the same conclusion viz that organic substances were translocated along the phloem.

W. SCHUMACHER [592] succeeded in operating upon the leaf-stalks of *Pelargonium* in such a way as to remove the circle of collateral bundles, though retaining the one large bundle. Subsequently the removal of nitrogenous substances from the leaf was ascertained microchemically. It was found that under such conditions about 75% of this removal still continued. Upon cutting of the xylem from this central bundle the removal of protein continued up to about 68%. When next the phloem was taken out as much as possible, this removal amounted to no more than 5%. Taking into account the extreme difficulty of these operations which can never be 100% accurate, these results may be regarded as conclusive and irrefutable proof of translocation through the phloem. SCHUMACHER further succeeded in causing a highly diluted solution of eosin to be drawn up in the sieve tubes and found that the parenchyma of the phloem remained totally uncoloured and to all appearances still quite normal, though the sieve tubes were coloured. Under those conditions all subsequent removal or organic substances is halted, from which it must be concluded that in this object translocation takes place through the sieve tubes, but not along its parenchyma.

E. J. MASKELL [411] and T. G. MASON [412], who studied the movement of carbohydrates and proteins in the stems and leaf-stalks of the cotton plant, *Gossypium herbaceum* in Trinidad, used quite different methods. They worked with a large number of cotton plants of the same strain, the same age, and grown in the same soil. They determined the carbohydrate and protein content in the different parts of these plants, so as to calculate the average value and the average error in a number of groups of ten plants each. The calculation of carbohydrates was based on the dry weight less the carbohydrates present. The authors correctly assumed that this figure was far less variable than that of the total dry weight.

Because MASKELL and MASON had a large amount of experimental material at their disposal, they were able to take samples of the leaves, the bast, the wood, etc., every 4 hours. The interesting part of this investigation was the study of correlation between the values found in the different organs. I cannot go into the details of this study of correlation, but will mention only its results:

(1) the daily variation of the total concentration of sugar in the leaf revealed a far greater correlation with that of the cortex than with that of the wood of the stem;
(2) the changes in concentration in the cortex follow those in the leaf after a certain time-lag;
(3) the changes in concentration of carbohydrates in the cotton plant are due mainly to changes in sucrose.

From these three points the authors concluded that the carbohydrates in the cotton plant are transported in the form of sucrose and that such translocation occurs through the cortex. It appeared from the correlation values that ringing causes an interruption in transport, but this was not the case when wood and bast were separated by the insertion of a sheet of paper. From these facts no other conclusion could be drawn than that translocation of sugar occurs through the bast.

In a later publication the authors relate how the cortex was divided into different layers, the inner layer with especially sieve tubes, and the outer one with the parenchyma. In this case they found that there was most correlation between the daily variation in the leaf and that in the layer with sieve tubes. This concerns the sucrose content while correlation with monoses was negative. Therefore, carbohydrate transport occurs in the sieve tubes and in the form of sucrose.

When MASKELL and MASON studied the translocation of nitrogenous substances with the same method, they concluded that this also takes place along the bast and in the sieve tubes. In regard to the substance translocated, they were of opinion that this was the so-called nitrogen residue, i.e. the total nitrogen less proteins and asparagine. The cotton plant does contain asparagine but only as a storage product, amino-acids being the substances transported.

Like SCHUMACHER in his experiments referred to above, MASKELL

and MASON also found velocities of translocation which were higher than could have been obtained by ordinary diffusion.

All these facts completely justify the statement that transport of organic substances in the higher plant passes through the sieve tubes of the phloem, but the question arises anew what is the cause of the great velocity and capacity of the flow.

As an attempt to solve this difficulty the work of E. MÜNCH [444] undoubtedly has great merit. This author first made a preliminary statement in 1926, but his book, "Die Stoffbewegungen in der Pflanze," the movements of substances in the plant, did not appear until 1931. The starting-point of MÜNCH's observations was the following experiment. In a tank of water he placed two osmotic cells of PFEFFER A and B, connected by a narrow tube, A having a high, B a low concentration of sugar. Water was strongly attracted into A and entered, and as a result of the resilience of the wall was pressed into B through the narrow tube. By itself B, osmotic cell with low concentration, would have a little water intake, but by virtue of the strong pressure of water through the narrow tube connecting A and B, water expulsion actually occurred in the cell with low concentration.

The important point of this experiment is that there will not only be diffusion-flow in the connection tube, but as a result of the plasticity of the wall a gradient ensues, which causes a mass-flow of the liquid in the tube, this mass-flow being incomparably more rapid than diffusion movements. This velocity is the greater as the diameter of the tube is smaller, although according to POISEUILLE's formula the increase in resistance is inversely proportional to the 4th power of the diameter.

MÜNCH considered that these observations, which can be traced back to PFEFFER [504], could be applied to the movement in the sieve tubes. The osmotic cell with high concentration represented the parenchyma of the leaf, where carbohydrates are formed as a result of photosynthesis, and the osmotic cell with low concentration was some growing tissue where sugars are consumed or some storage organ, where sugars are converted into starch. The connecting tube represented the sieve tubes; the dead vessels of the xylem containing water, could be likened to the tank in which the osmotic cells were placed. Thus, according to MÜNCH, the conditions under which the mass-flow translocating sugars through the sieve tubes occurs, were realized.

At first sight this sounds convincing, but on closer consideration it becomes obvious that the connecting tube of the physical experiment is quite different from the transport route in the plant. MÜNCH had to assume that there were permeable partitions in the connecting routes, through which the solution would be forced. Such cross-partitions, says MÜNCH, will no doubt offer a certain amount of resistance and cause higher hydraulic pressure in A, but for the rest the flow occurs in the same way as that without obstacles. But the question arises whether

this is not too simple a view and whether, perhaps, the resistance ultimately becomes so great that there is no longer any question of mass-flow.

Not a single cell of the spongy and the palisade parenchyma borders directly on any sieve tube, so part of the connecting routes would have to be formed by parenchymatous cells, in which case a sugar translocation would meet with obstacles of quite a different nature.

MÜNCH realised this difficulty and tried to overcome it as follows: According to him the transport route was much wider in the parenchyma cells, on account of its having to supply the sieve tubes on all sides; moreover, in his opinion transport took place through the connections between the living protoplasts, the so-called plasmodesms. Tests with dyes, however, never revealed any trace of a flow through the plasmodesms. The width of these pores in the cell-wall is estimated at 0,5μ and the resistance in such apertures has to be considered with a view to the question whether a more or less viscous liquid can be forced through them with the excess pressure available.

If there is indeed a flow under pressure in the sieve tubes, it might be expected that a cut would cause a clearly discernible exudation of sap. In 1860 TH. HARTIG [239] found that incisions made into the bast of the oak, the beech and the lime tree in summer produced a few drops of sap, containing nitrogenous substances, sucrose and in the case of Oleaceae such as the manna ash also mannitol. Except with the Oleaceae, the exudation only lasts a very short time and after a second incision is no more than a result of the local cell-wall tensions. The fact that the exudation soon ceases does not fit in with MÜNCH's considerations and he, therefore, fell back on the assumption expressed by A. FISCHER [174] in 1885, that a slimy mass must have been pressed against the sieve-plates, causing these to become blocked. But is not this at the same time an argument against the entire mass-flow theory?

Another point meriting closer consideration is the comparison of what occurred in B in the experiment. In the growing cambium there must be a conversion of sugar into insoluble cell-wall substances, and according to MÜNCH the hydrostatic pressure in the sieve tubes causes water to be expelled. He considers that in normal circumstances this water is taken up again in the adjoining xylem, but when the wood and the bast of a living branch are separated, there should be an observable exudation of water on the inside of the bast.

The results of the experiments in this matter are contradictory. As already stated, the difficulty lies in the fact that in every case water-vapour is exuded, whilst only secretion of fluid water would prove MÜNCH's theory. His own experiments are open to criticism and whilst a repetition by some investigators led to positive results, only negative ones were obtained by J. WESTENBERG [713] and the present writer.

All these considerations were probably the cause of the theory

meeting with scant approval from prominent German physiologists such as L. JOST [305] and H. FITTING [178].

So the mass-flow theory is based on only a few experiments, its strength being derived rather from circumstantial evidence, giving prominence to various facts which more or less fit in with the theory.

MÜNCH is of the opinion that in spring cambial growth begins in the roots and that the water which is liberated in the process is transported to the wood ducts and causes the root pressure. Apart from the quantitative aspect of the question, it should be noted that he thus assumes a connection between root pressure and cambial activity of the roots, which connection has so far by no means been proved. Earlier botanists of the first half of the previous century such as HUGO VON MOHL [435], stated that cambial root growth of the oak and the ash may be observed at a time when there is still no question of root pressure. Experiments carried out by the present writer in the socalled "root house" of the laboratory for plant physiology at Wageningen revealed that in the birch root growth occurred long before there was any question of bleeding.

A kind of compromise between the mass-flow theory and other ones was proposed by A. S. CRAFTS [124]. Diffusion along plasmodesms of cross walls and acceleration by protoplasmic streaming within non vascular tissues might be combined with pressure flow through permeable sieve tubes. This mass-flow would even pass through phloem walls longitudinally; a very strange notion in my opinion. In this hypothesis CRAFTS assumed a rather high concentration of sugars in the sieve tubes; at least a 10% solution in the developing potato tuber. This assumption, however, does not apply to other objects. As early as 1865 was shown by SACHS that the dry matter in the sieve tubes of *Cucurbita* spec. is largely of a nitrogenous nature and contains very little sugar. Recently this was completely substantiated; in different objects sugars constitute about 0,5% of the fresh weight.

In the case of ripening fruits MÜNCH assumed a mass-flow through the phloem consisting of a diluted solution of sugars and nitrogenous compounds. After depositing these substances a transport of water would take place through the xylem in the inverse direction. MÜNCH's assumption was tested by H. F. CLEMENTS [119] with an interesting object, the fruits of the sausage tree, *Kiggelaria africana*. The assumption involved the movement of such tremendous quantities of water through poorly adapted tissues" says CLEMENTS that MÜNCH's hypothesis was found to be inadequate. No evidence could be obtained of the return of water from the fruit through the xylem tissue.

There are investigators who are opposed to the mass-flow theory on the ground of entirely different experiments. Of these SCHUMACHER [592] in particular should be mentioned. This author studied the subject in petals of so-called ephemeral flowers. Various Cactaceae, Orchidaceae and Convolvulaceae have flowers which wilt very quickly

especially after pollination. In general a slight decrease of proteins may be observed before wilting, but in ephemeral flowers there is an extremely rapid removal of N-compounds after pollination. For example in *Hydrocleis nymphaeoides*, one of the Butomaceae, 42% of the proteins in the perianth disappear in the space of two hours. SCHUMACHER found that 67% of the nitrogen-content of the flower was translocated to the rest of the plant, mainly during the period when the turgescence of the organs removing the nitrogen had gone. It stands to reason that this is completely at variance with the mass-flow theory, for this mass-flow requires the turgescence of the tissue.

Another investigation is based on the use of fluorescent dyes, which are taken up into the living tissues and then followed on their path with the aid of the fluorescence microscope. SCHUMACHER succeeded in making such experiments with *Pelargonium zonale* by scraping off the epidermis and subsequently putting fluorescein-potassium on the surface of the leaf. This enables the transport to be observed in the protoplasm. The walls do not show the fluorescence, nor does the vacuole. As regards the vacuole, however, it may be that the degree of acidity is the cause of non-fluorescence. It was found that the velocity amounted to approximately 30 cm. per hour, a value which more or less agrees with that found by SCHUMACHER in his investigation regarding the velocity of translocation in the sieve tubes of *Pelargonium*, to which reference has been made above. It was clear that the translocation of fluorescein was partly due to a gradient, and the flow was halted by cooling. To all appearances narcosis does not have this effect, but it is not certain that in those experiments a state of narcosis did in effect supervene. Undoubtedly SCHUMACHER obtained interesting data by this method.

MASKELL and MASON also raised objections to the mass-flow theory. They again worked with the cotton plant in the manner already described and simultaneously studied the transport of sugars and nitrogenous substances. They found that this transport was governed entirely by differences in concentration. By means of experiments, which cannot be described here in full, they succeeded in regulating the concentrations of both kinds of substances in such a way that carbohydrates and nitrogenous substances could be transported in one and the same stem in opposite directions. This is surely another argument against the mass-flow theory, though MÜNCH could defend the latter with the argument that there may be totally independent transport routes in one and the same stem, as was indicated by ROUSCHAL's [555] experiments with fluorescein.

In the view of the present writer it is best not to regard the mass-flow theory as irrefutably proved. Contrary to the arguments against the theory, raised by the physiologists above-mentioned, are the results of investigations carried out in the laboratory of Prof. W. H. ARISZ [13] at Groningen, Particularly the work of M. P. BOTH [64] pleads in favour of the mass-flow theory. The case has not been settled yet, and it seems

advisable to take into consideration also the different views presented during the past few years.

In the first place we must again consider the question whether translocation of organic substances is possible along a narcotised leaf-stalk. This question was already referred to in the discussion of CZAPEK's work. It will be remembered that the conclusion drawn by this author from his experiments was that such translocation is possible only in actively living tissue. DELEANO's work had thrown doubts upon this conclusion, so W. M. KRUSEMAN [351] repeated the experiments in the laboratory of F. A. F. C. WENT [709] at Utrecht. He found that narcosis by means of chloroform did, in fact, inhibit the translocation, hence he concluded that the living protoplast must exert some influence on the process. S. J. DIJKSTRA [151] confirmed these results of KRUSEMAN and stated the influence of oxygen tension.

The work of MASON and PHILLIS [506] with the cotton plant also supported the assumption that living tissue is essential in the translocation of organic products. These authors particularly studied the question as to whether oxygen tension is of any importance to this translocation. They observed that by covering the leaf-stalk with wax the removal of carbohydrates was first inhibited, though later the original condition prevailed. The authors attributed this phenomenon to an admission of oxygen by way of the intercellular spaces. A lowering of the oxygen tension in the neighbourhood of the leaf-stalk was found to have an inhibitory effect at times, though not at other times, and this also they ascribed to an admission of oxygen through the intercellular spaces, although this rather weakens their case.

More convincing are the experiments in which they separated the bast from the wood and covered both sides of the former with vaseline. Their conclusion was that respiration is a "conditio sine qua non", for the translocation, i.e. translocation is a function of the living protoplasm.

In his above cited publication CLEMENTS concludes that the observed movement of sugars into the fruit of *Kiggelia africana* is so great that it seems necessary to describe it as a function of the living protoplasm of the sieve tubes which through its respiration activity does influence the movement.

So we must obviously once more return to our starting point, the co-operation of protoplasmic streaming as argued by HUGO DE VRIES. CURTIS [128] did the same, and felt compelled to reject the arguments of E. STRASBURGER [636] by stating that, though the latter established that such streaming did not occur in preparations of the sieve tubes, this settles nothing in regard to the question whether the streaming occurs in intact sieve tubes "in situ." This is undoubtedly correct, though it no more proves that such streaming does occur in the intact sieve tubes. In the discussion on FITTING's [178] investigations with *Vallisneria* we shall see later how extremely sensitive this protoplasmic streaming is.

From wounding experiments with cut off leaves F. W. WENT [710] quite recently concluded that, leaving all theories apart we must take due note of the fact that inside the sieve tubes a high turgor pressure exists.

Finally mention must be made of a publication by T. H. VAN DEN HONERT [267]. This author's starting point is the fact that a substance which lowers the surface tension quickly spreads itself over the surface of another liquid, forming a monomolecular layer thereon, a film with the thickness of one molecule. This may also happen if such a substance which lowers the surface tension extends itself over the interface between two liquids which do not mix. In demonstrating this with a special apparatus, VAN DEN HONERT showed that a streaming of liquid may ensue, but this does not answer the question whether this idea is of any practical significance with regard to the movement of carbohydrates and amino acids in the sieve tubes.

In the present writer's opinion, the fact that various investigators have revealed the dominating influence of respiration on this process would seem to argue against a conception in the sense of VAN DEN HONERT as well as against the mass-flow theory. The problem as to what causes the streaming in the sieve tubes still awaits its ultimate solution.

Chapter VII

METABOLISM OF HETEROTROPHIC PLANTS, INTAKE OF ORGANIC NUTRIMENT

(A) General Observations on Metabolism.

The metabolism of heterotrophic plants is a subject not easy to review. At first sight it seems as if our views have not undergone any fundamental changes during the past few decades, but on closer consideration this is found to be incorrect. It was not until the 3rd edition of the Textbook by Hugo de Vries appeared that the metabolism of Fungi and bacteria began to be really understood. At present we can look back on an enormous amount of work done, biology has grown an entirely new branch, bacteriology, which is an independent science with its own methods and its own goal. Here more than in other chapters I shall have to confine myself to a selection from a vast body of research; it is evident that bacteriology as a whole cannot be treated of here.

Nor is it a simple matter to consider the innumerable publications from only a single point of view. I will not attempt to do so, because firstly, a number of investigations come within the category of nitrogen metabolism and have already partially been dealt with in Chapter V, and secondly most of the investigations concern processes of dissimilation of heterotrophic plants and are closely connected with the discussion on respiration and fermentation in Chapter II.

In the University of London my countryman A. J. Kluijver [331] gave in 1930 a series of lectures on this subject, which have since appeared in book form under the title "The chemical Activities of Micro-Organisms." In these lectures the general aspects are set forth with admirable consistency and practically all the processes of dissimilation, carried out by micro-organisms, are considered from the point of view of hydrogen activation.

Conversions such as that of sugar into ethyl alcohol, acetic acid, lactic acid and butyric acid, are regarded as more or less complex dehydrogenations and hydrogenations of the following types, linked together in the living cell:

I. $AH + B \rightarrow A + BH$ e.g. $AH_2 + O_2 \rightarrow A + H_2O_2$
II. $AH.B \rightarrow A.BH$
III. $AH.B \rightarrow A + BH$
IV. $AH + B \rightarrow A.BH$

Too much space would be required to enter into details, though it must be pointed out that in 1930 KLUYVER brough into relief the enzymatic character of many, if not all, dehydrogenations of the processes of dissimilation, which can be reproduced in vitro. It is questionable, however, whether this is true of all these processes and whether a distinction should still be made between enzymatic and non-enzymatic biochemical processes.

According to one school the non-enzymatic biochemical processes are the most typical metabolic processes which occur only in the intact, living cell. Thus we again encounter here the old distinction between plasmatic and aplasmatic metabolism. C. OPPENHEIMER [477] made a distinction of this kind in his textbook, but in view of what has been said in Chapter II this can surely be maintained no longer in respect of dissimilatory processes. KLUIJVER suggested the possibility of a distinction being made between dissimilatory processes on the one hand, and synthetic processes, on the other. The view that synthetic processes are not voluntary reactions might warrant such a distinction, but on closer consideration this, again, is not tenable.

There is no reason to assume that the oxido-reduction processes, which give rise to new and more complicated carbon chains in the way discussed above in Chapter V in connection with the synthesis of fats would differ in principle from other oxido-reductions. For instance, NEUBERG [456] showed experimentally that the condensation reaction forming ethyl alcohol has an enzymatic character.

Nevertheless it has till now not proved possible synthetically to form fats from sugars because of the fact that syntheses require the harmonic co-operation of a number of reactions which apparently can only realized in the living cell; co-ordination of such processes is the most essential element of life.

KLUIJVER winds up his observations with a question. "should a living cell be considered as an arsenal filled up with enzymes, which successively are brought into action? Such a supposition would only be justified if every chemical reaction brought about by the cell required its own specific catalyst." The specificity of the enzymes with hydrolytic action has led several investigators to assume the same specific functions in respect of oxido-reductases or dehydrogenases. However KLUIJVER points out that this cannot be correct in view of the results obtained by his pupil L. E. DEN DOOREN DE JONG [143], who found that *Pseudomonas putrida* was capable of utilizing a very large number (80) of different organic compounds — experiments were made with about 200 — as sole source of carbon. To assume the presence of an equal

number of different dehydrogenases in the cell of *Pseudomonas putrida* is, of course, absurd, the more so because artificial products such as bromo-succinic acid or bromo-propionic acid can also be utilized. There is no doubt a certain degree of specificity, but KLUIJVER regards electrostatic factors as the main point. It is a case of the uncoupling of valences, and since nowadays chemical binding is regarded as being electrostatic in character, activation by means of the enzymes is also ascribed to what may be a polarizing action of its electric field, the specificity of the enzyme being the result of a difference in intensity of this field.

In this connection KLUIJVER also considers the important question as to what is exactly the significance of oxido-reduction in the living cell. In other words, what is the purpose of dissimilation, a question also suggested in Chapter II. Reference is made here to the research of A. V. HILL [257] regarding the consumption of oxygen in animal tissues in a resting state.

HILL found that the function of the oxido-reduction processes is the preservation of a membrane-potential around the tissue, which in its turn is essential for the maintenance of the dynamic equilibrium in the cells. KLUIJVER concludes that not only are oxidation-reduction processes essential for the maintenance of differences in potential, but the existence of such differences is an indispensable condition for, even the cause of, oxido-reduction. Perhaps we shall come to the conclusion ultimately that "the biological master key which is responsible for all primary oxido-reductions underlying metabolism is nothing but a protein with an electric double layer which is characterised by a pronounced difference in potential. Here we might also find the link which evidently exists between the processes of oxido-reduction on the one hand and of protein breakdown on the other. The very maintenance of this potential difference by the oxido-reduction processes would then protect the protoplasmic micelle against the continuously present menace of autolysis."

The old adage "Natura non facit saltum," which freely translated means, "Nature does not know any sudden transitions," also holds good for heterotrophic plants. A multicellular organism such as the higher plant is autotrophic as far as some cells are concerned, for the cells containing chlorophyll form all their constituents from inorganic materials with the aid of sun-energy. But the non-chlorophyllous cells are heterotrophic and derive their building-materials from the organic products formed in the cells with photosynthesis. In view of this it is not surprising that a unicellular Alga, *Chlorella variegata*, is autotrophic in its green cells, whereas the variegated variety can exist only when carbohydrates are supplied and is therefore, a heterotrophic organism.

Thus it also becomes understandable how Belgian and French workers, E. LAURENT [365] and J. LEFÈVRE [369] were able to grow seedlings of maize and garden-cress, *Lepidium sativum*, in an atmosphere without carbon dioxide if the roots could take up sugars and amino acids under

aseptic conditions. Apparently there are no fundamental differences between autotrophic and heterotrophic plants.

Speaking generally, it may be said that both groups require the same elements. Recently a number of investigations into this matter were made with saprophytic, heterotrophic plants which are pre-eminently suitable for such work. It will be remembered that in Chapter IV the essentiality of, for instance, magnesium was demonstrated by experiments on the heterotrophic Fungus *Aspergillus niger*. During the present century WATERMAN [702] in particular, already mentioned in these pages, experienced in this direction on the basis of the work of his teacher M.W. BEIJERINCK [48]. Forin stance, Waterman found that traces of zinc sulphate may reduce the essential quantity of magnesium to one-tenth of the quality normally required. All the same there are certain differences between a saprophyte such as *Aspergillus niger* and higher plants as regards the essential elements. Calcium, if really essential-which is very difficult to decide — is required only in minute quantities. This has probably some connection with the fact that the calcium compounds of pectic acid form an important part of the cell-wall of higher plants, though they are lacking in the chitinous walls of Fungi.

It has also become evident during the present century that many heterotrophic plants are characterized by a rare skill in the use of all kinds of organic substances for the formation of their protoplasm, on the one hand, and for the acquisition of the necessary energy on the other. There are micro-organisms which can build up their entire substances from methylamine, CH_3NH_2. It is almost possible to say that there is not a single natural substance which cannot be utilized by some heterotrophic organism as a source for its carbon. The facts mentioned above about *Pseudomonas putrida* are a proof of this thesis .Even the paraffins, which, as their name indicates, are very stable compounds, can be attacked by micro-organisms.

It is now recognized that PFEFFER's list of organic substances, including sugars, peptone, quinic-, tartaric-, and citric acids, asparagine, acetic acid, lactic acid, ethylalcohol, benzoic acid, methylamine, phenol, in descending order of usefulness as sources of carbon nutriment, is quite incorrect and obsolete. Investigations such as those of WATERMAN have since proved that a substance may be both a source of energy and a poison, though this depends on its concentration. For instance, phenol is a better source of nutriment in an extremely low concentration than in a high one.

It is obvious that this ability to use all manner of substances as nutrients has some connection with the dehydrogenation processes mentioned above. The reverse is the fact which PASTEUR [497] observed in the past century, that of two stereo-isomeric organic substances such as dextrotartaric and laevotartaric acid, the former is a much better nutrient for *Penicillium* than the latter.

That the metabolism of heterotrophic plants depends to a considerable extent upon the enzymes formed by them became known through an investigation by F. A. F. C. WENT [709] at the beginning of the present century. In *Monilia* this author found, apart from the respiratory enzymes, some ten others which may bring about hydrolysing reactions. Apparently the secretion of these enzymes can be adjusted to some extent to suit the metabolic circumstances. With *Aspergillus niger* G. L. FUNKE [189] observed that diastase appears in the culture solution to a lesser extent if the concentration of soluble sugar, for instance sucrose, rises above a certain value but this author considered there was a possibility that the diastase, though formed, undergoes temporary binding.

A matter for closer consideration is whether there are any heterotrophic organisms which can use nitrogen as a gas and not in a chemical compound for the synthesis of their protoplasm. Such a question is evidently of great importance to agriculture.

In 1895, when the 3rd edition of the Textbook appeared, experience had shown that there must be bacteria in the soil which are able to fix nitrogen, though their isolation had not then been established.

The pioneering work in this respect was done by S. WINOGRADSKY [735] in 1895, when he isolated from the soil the bacterium *Clostridium Pasteurianum*, which carries out the fermentation of butyric acid in an anaerobic environment and can derive its nitrogen compounds from molecular nitrogen. In the soil this is possible even in the presence of oxygen, provided that in the neighbourhood of *Clostridium Pasteurianum* there are other species of bacteria with a strong affinity for oxygen.

A few years afterwards BEYERINCK [48] discovered the existence of bacteria that live in an aerobic environment and also fix and assimilate molecular nitrogen, *Azotobacter chroococcum* and *Azotobacter agile*. For their early development these require traces of nitrogen compounds, but at a later stage they are capable of fixing molecular nitrogen much more intensely than *Clostridium*. With both, *Clostridium* and *Azotobacter* the presence of ammonium or nitrates diminishes the rate of nitrogen fixation, at least at a later stage.

Some of the Cyanophyceae, or blue green Algae, are as well capable of carrying out this process of nitrogen fixation. Recently it was shown by ALLISON [5], HOOVER [272] and MORRIS [441] by application of the trace technique with the isotope N15 that the blue green Alga *Nostoc muscorum* as well as *Azotobacter* and *Clostridium* fixed readily determined quantities of molecular nitrogen.

This process is of tremendous importance to other organisms, all of which, but for a few exceptions can derive the nitrogen they require only from compounds of this element. Since part of such compounds seem to yield molecular nitrogen again in the course of their breakdown in metabolism, it would be lost to the plants, without the

nitrogen fixation of these organisms. It is true that electric discharges during a thunderstorm, particularly in the tropics, assist the formation of nitrogen compounds from molecular nitrogen, but this is not sufficient.

We have still got to consider a point which is of great importance to physiology, viz. the question how this nitrogen fixation takes place. Some investigators such as S. KOSTYTSCHEW [344] expressed the view that ammonia is the primary product, and according to WINOGRADSKY who returned to the study of this subject in his later years, the appearance of ammonia in pure cultures indicates a direct reduction of molecular nitrogen. Others considered the possibility of the formation of N_2O_3 compounds with iron as a catalyst, but this again, is purely hypothetical. Time and again the idea cropped up that there might be direct binding of hydrogen. This would be in keeping with the view that activated hydrogen plays a part in dissimilation. In the fermentation of glucose, for instance, *Clostridium* produces hydrogen in addition to butyric acid and carbon dioxide.

With *Azotobacter* nitrogen fixation ceases as soon as the oxygen content in the environment becomes too high, and with some species the optimum fixation occurs in an atmosphere with 1% oxygen. In 1930 H. BORTELS [62] made the interesting observation that traces of the element molybdenum are essential for this process, and it is possible, therefore, that the binding of hydrogen and nitrogen occurs with the aid of a catalyst containing molybdenum. If this is so, a comparison with the technical process discovered by HABER [221] for the synthesis of ammonia from a mixture of nitrogen and hydrogen with the aid of the catalyst uranium by induction sparks or dark electric discharges becomes obvious. D. BURK [96] and H. LINEWEAVER [379] demonstrated that a fair amount of calcium was necessary for the binding of nitrogen. BURK and HORNER [276] maintain that the ammonium production of *Azotobacter* is largely independent of the form of nitrogen supplied. BURK suggests that a specific enzyme, nitrogenase, is responsible for this nitrogen fixation in *Azotobacter*; ammonia might arise in a secondary manner by oxydative disamination.

In the Annual Review of Biochemistry of 1944 BURRIS [98] and WILSON [733] mention their investigations in which the isotope N^{15} was used to determine the effect of combined nitrogen on nitrogen fixation by *Azotobacter*. Adaptation by previous culture in a medium containing nitrates was necessary before this form of combined nitrogen completely suppresses fixation. According to these authors the studies with isotopes favour the hypothesis of the primary formation of glutaric acid and by implication ammonia.

(B) Root Nodules.

Progress has been made in more recent years concerning nitrogen fixation in root nodules. In 1888 H. HELLRIEGEL [248] and WILFAHRT [731]

concluded that the Papilionaceae were capable of fixing nitrogen, provided that there were nodules on the roots, which had arisen as a result of infection by organisms in the soil. BEIJERINCK advanced a step further when he demonstrated that the organism, now termed *Bacterium radicicola* or *Rhizobium leguminosarum*, introduced in the roots in pure culture, is the cause of the nodule formation and nitrogen fixation. All efforts, however, to effect nitrogen fixation in a pure culture of these organisms at first met with failure. During the past few decades fairly good results were sometimes obtained by F. E. ALLISON [5] and S. R. HOOVER [272] when the raw materials were less thoroughly purified, and it seemed possible that some bios substance or other might play a part here. Heterauxin production by the bacteria seemed to be cause of the reaction shown by infected roots, a fact observed by THIMANN [649] in 1936. WILSON, HOPKINS [274], and Fred [184] studied nitrogen fixation by *Rhizobia* apart from the host plant. Recent investigations have proved that the chief growth factor for the root nodules is biotine, a bios substance which will be dealt with later (Chapter X).

A. I. VIRTANEN [682] succeeded in obtaining a fixation of nitrogen in pure culture by the addition of oxalacetic acid. He associated this with a theory of the chemistry of the nitrogen binding, which is as follows. In his view hydroxylamine, NH_2OH, is formed in the bacteria which with the oxalacetic acid formed by the metabolism of the Papilionaceae combines to form asparagine, an intermediate stage in this process being an oxime which may yield aspartic acid by hydrogenation.

$$NH_2OH + HOOC.CO.CH_2COOH \rightarrow HOOC(NOH.CH_2COOH + H_2O$$

$$HOOC.C(NOH).CH_2.COOH + 4\,H \rightarrow HOOC.CHNH_2.CH_2.COOH + H_2O$$

Though this has not been strictly proved, there is some circumstantial evidence, firstly the fact already mentioned that bacteria in pure culture can carry out the nitrogen assimilation only if they are supplied with oxalacetic acid; secondly VIRTANEN was able to isolate oxalacetic acid from pea plants; and thirdly it was possible to demonstrate the presence of the oxime of this acid, which may arise as the result of the action of hydroxylamine on oxalacetic acid in the bacterial nodules. Finally, by hydrogenation this oxime may yield aspartic acid, which is an excellent source of nitrogen for Papilionaceae in water culture. Furthermore VIRTANEN observed that the bacterial nodules of the Papilionaceae in some cases liberate organic nitrogen compounds, in this case aspartic acid and alanine, of which the latter may have arisen by the splitting off of carbon dioxide from aspartic acid. This last-named fact, the liberation of such nitrogen compounds by the living nodules, brings about a stronger development of plants in the immediate surroundings and confirms the evidence of ancient cultures.

It must be stated, however, that American workers were unable to

confirm VIRTANEN's findings, at least to obtain nitrogen excretion by Leguminous plants of the order of that observed by this author. Recently H. KUBO [352] made the interesting discovery of haemoglobin in root nodules.

Is it possible to reconcile the ammonia and hydroxylamine hypothesis of nitrogen fixation? The experiments with the isotope N^{15} were primarily made with *Azotobacter* and the excretion experiments with Leguminous plants. Other studies, however, pointed to a close similarity in the fixation mechanism, so the choice of agent is not responsible. WILSON's suggestion is that the two compounds, may be part of the same mechanism in which the precise pathway eventually is dictated by the availability of the carbon chain functioning as the acceptor of fixed nitrogen. Biological fixation of nitrogen is specifically inhibited by hydrogen. WILSON c. s. established this fact both in *Azotobacter* and *Nostoc* as well as in root nodules. Carbon monoxide inhibits the fixation by red clover in very low concentrations (0,0001-0,0005 atmosph.), by *Azotobacter* it is about tenfold these concentrations. This specific inhibition by both hydrogen and carbon monoxide indicates a considerable unity in the enzymatic mechanism of nitrogen fixation. The presence of the enzyme hydrogenase in *Azotobacter* has been reported; sometimes also in bacteria taken from the nodules. Formation of hydrogenase in *Azotobacter* appears more responsive to the presence of nitrogen than that of hydrogen. WILSON concludes that the enzyme is largely associated with the nitrogenfixing system of this organism.

A few words must be said about the rhizothamnia of *Myrica Gale, Alnus glutinosa, Hippophae rham noides*. NOBBE [463] and HILTNER [259], later SCHIOESING and LAURENT established fixation of atmospheric nitrogen. The opinions concerning the nature of the organisms which cause the formation of these rhizothamnia are contradictory. The greater part of the workers in this field regard the organisms as *Actinomyces*, and R. SCHAEDE [572] speaks of an Actinomyces symbiose in *Myrica Gale*, the sweet gale. He stated that there are two kinds of nodules, in one kind the host plant dominates the endophyt which is finally digested, in the other kind *Actinomyces* forms bacteroids.

Observations regarding the bacteria in the leaves of the Myrsinaceae, particularly in those of the genus *Ardisia*, have also been made during the present century. H. MIEHE [429] was the first to examine these in 1911; later they were subjected to closer study by F. C. VON FABER [168], who both isolated the bacteria and grew the plant in their absence. Under such circumstances the development of the plant is found to be greatly inhibited. In connection with the allegation that the bacteria used the nitrogen out of the atmosphere, this fact gave rise to the hypothesis of nitrogen fixation by one symbiont on behalf of the other.

During the past few years this problem has been considered further by Ph. DE JONGH [304] in the laboratory for plant physiology at Leyden.

The theory of nitrogen fixation was not confirmed, and it appeared that the presence of the bacteria in the leaves is of little importance to *Ardisia* though their presence in the growing points is important. The formation of the ovules occurs in the growing points of the flowers, so that the bacteria are enclosed in the seed. If these bacteria are eliminated by heating the seed to a temperature of 40°C for about 24 hours, it will to all appearances out come undamaged, but when germinated a dwarf with only a few leaves and no flowers will develop, instead of a normal plant. Cytologically the plant is normal, but there is no differentiation of the leaf primordia and no growth of the organs.It does seem to be possible, however, to make these dwarfs grow into normal plants by infecting them at a very early stage with the bacteria that were isolated. The action of the bacterial infection is still unknown. Hence this case is quite different from that of the Papilionaceae. In *Ardisia* the presence of the bacteria is essential to normal development, though this is not so with the Papilionaceae.

With Leguminous plants, when the relation of the bacteria in the nodules to their host is examined, it is found that there is a kind of equilibrium between these two parties. This equilibrium may be broken under the influence of different circumstances. Thus, if the host does not grow sufficiently, the bacteria turn into parasites which feed at the expense of their host. Vice versa, it may happen that strong development of the host may lead to the bacteria being killed and consumed by the host plant, with the result that the latter benefits by the nitrogen compounds obtained by assimilation of the nitrogen from the atmosphere.

This is a clear example of what used to be termed symbiosis, but what in the present writer's opinion might be more correctly called mutual parasitism. The former term, which is sometimes more closely defined as mutual symbiosis usually denotes a living together to mutual benefit. But though this may appear to be true at first sight, from what has already been observed the term mutual parasitism would seem to be more appropriate. Hence the state of affairs in the Leguminous plants may be described by the term optional symbiosis or optional mutual parasitism, whereas in *Ardisia* obligatory symbiosis is encountered.

(C) Mycorrhizas.

The term symbiosis, which was first applied in 1879 by H. A. DE BARY [32] to describe the association of Alga and Fungus in Lichens, was also used by A. B. FRANK [182], who a few years later described the different kinds of mycorrhizas or the associations of certain species of Fungi with the roots of plants.

FRANK made a distinction between the ectotrophic mycorrhiza, where the mycelium is largely outside the root tissue and only penetrates

between the cells of the endodermis, and the endotrophic mycorrhiza with intracellular hyphae. The latter case was first described by M. J. SCHLEIDEN [576] in connection with the bird's nest orchid, *Neottia nidus avis*, while the former was first observed by KAMIENSKY [309] in *Monotropa hypopitys*.

Between mutual symbiosis and the parasitizing of one symbiont upon another there are various intermediate stages, whilst besides species with permanent mycorrhiza there are those where this is more or less accidental.

Views on the significance of mycorrhiza were so divergent that in 1900 E. STAHL [620] published a book in which he made a distinction between obligatory and optional or facultative mycorrhiza. According to STAHL there was some connection between mycorrhiza and the struggle to obtain nutrient salts, which, especially in humus, was very fierce between Fungi and higher plants. He considered that as a result of the development of mycorrhiza the Fungus became the servant of the higher plant instead of being its rival. Though there may be some point in this anthropomorphistic view as far as plants with ectotrophic mycorrhiza are concerned, it is an utterly unlikely assumption in regard to those with endotrophic mycorrhiza on account of the fact that its hyphae have but little connection with the outer world. STAHL tried to help himself out of the difficulty by assuming that in these cases the Fungus had the function of assimilating the inorganic substances, but there is no ground whatever for this assumption.

At the beginning of this century some painstaking observations concerning endotrophic mycorrhizas were published. W. MAGNUS [403] examined *Neottia nidus avis* and K. SHIBATA [603] studied *Psilotum triquetrum*, one of the Pteridophytae. In the second case the Fungus, which penetrates from outside, develops in the cortex of the roots into two types: in one of these the hyphae remain only on the periphery of the cell, whilst the nucleus retains its normal character, and in the other there arises in the cells a dense ball of hyphae which later tends to show symptoms of disorganisation, while the nucleus becomes enlarged and takes on the appearance of the nucleus of a gland cell. Herein the clump of hyphae loses its protoplasm and only the chitinous cellwalls- of the hyphae remain, which clearly shows the parasitic action of the host. With this type the term mutual parasitism aptly describes the facts, for first the Fungus parasitizes on the host, but later the roles are reversed, whereas with the first-mentioned type the two symbionts seem to balance one another.

A comparison of the course of events in *Neottia* and *Psilotum* shows that the differences between them are not fundamental, but *Neottia* contains very little chlorophyll. Only in very young plants does the uptake of carbon dioxide by photosynthesis exceed the production thereof by respiration, so that the Orchid plant is almost entirely dependent upon the Fungus for its nutrition or, in other words,

parasitizes upon it. In *Neottia* the cells in which the Fungus is preserved are much more clearly divided in a separate layer from the cells in which the Fungus is resorbed than in *Psilotum*. In the resorbing cells the change in appearance of the cell-nucleus is so pronounced that some authors have compared these to phagocytes.

NOEL BERNARD [42] was the first to establish that the Fungus may also be an essential partner to green Orchids, which gave the problem a totally different aspect. Like BERNARD, H. BURGEFF [94] studied the problem of the germination of Orchids and the structure of their rhizocormia. The conclusion of both was that the roots of Orchids are inhabited by a special species of *Rhizoctonia* or *Orcheomyces* which is not harmful to its host. In the normal course of events germination of Orchids occurs only in the presence of the Fungus. BERNARD in particular stressed the fact that it is a typical mutual symbiosis, the Fungus obtaining its food from the Orchid, while promoting the latter's germination.

In regard to the mode of digestion of the Fungus, BURGEFF distinguished between tolypophagy and ptyophagy. In the former case the walls of the hyphae remain intact and the contents are resorbed, while in the latter the hyphae burst and the fragments are scattered in the host-cell. Ptyophagy occurs in some tropical saprophytic Orchids such as *Gastrodia* and also in the European Ericacea *Monotropa hypopitys*. The latter will be discussed later.

Diametrically opposed to BERNARD's view was that of L. KNUDSON [337], who studied the question of the germination of the green, tropical Orchids *Cattleya* and *Cypripedium* in 1925. This author's starting point was the observation that in these cases germination is possible even without *Rhizoctonia* or any other Fungus, provided that the environment contains a certain concentration of sugars and the p_H is below 5. According to him, the Fungus is only indirectly useful by forming sugar with the aid of its enzymes and by forming acids for the p_H required. His conclusion that this symbiosis was not obligatory was confirmed later in respect of tropical species of Orchids by K. G. QUEDNOW [524]. But the controversy was not yet settled.

Though BURGEFF admitted the correctness of KNUDSON's results, he regarded them as exceptions due to an unnatural state of affairs. Moreover, KNUDSON's conclusions do not hold good in the case of the green Orchids of West-Europe. Despite all precautions, these do not germinate well without Fungi, for the protocormia remain yellow and do not develop normally. In VERMEULEN's [674] experiments with *Dactylorchis* the protocorm only develops if a *suitable* medium is present and what is a suitable medium, which substances must be present? There is reason to assume that so-called bios substances, of which more later, play a part in the process. In species of the tropical Orchid *Vanda*, G. SCHAFFSTEIN [573] observed avitaminosis or a deficiency of bios substances when the seeds were raised

in a sterile condition on an artificial substrate. He termed this vitamin vandophytine and obtained it from all kinds of plants, after which it could be purified and concentrated. As nicotinic acid and its amide are capable of remedying the avitaminosis of Orchids, it is likely that these substances, which form part of dehydrases, are closely connected with vandophytine.

There has been a great deal of controversy concerning the mycorrhiza of the Ericaceae and even now its function is not quite clear. FRANK [182] had already observed that the thin roots consist of a narrow central cylinder, surrounded by a single layer of cells of large dimension and filled with hyphae. According to Mrs. M. C. RAYNER [532] symbiosis was permanent and obligatory and the parts above ground likewise contained hyphae, but others like L. KNUDSON arrived at quite different conclusions. Under absolutely aseptic conditions the last-named author obtained seedlings of the Scotch heather, *Calluna vulgaris*, from which fact he concluded that symbiosis could not be obligatory. It is in keeping with this view that in dry soil, poor in humus, the mycelium is sometimes entirely lacking.

R. FREISLEBEN [185] made similar observations with *Vaccinium myrtillus* under aseptic conditions. He found that, whereas in peatmoss soil the seedlings cease to develop, they continue to do so on a synthetic substrate, from which he concluded that in peatmoss soil substances occur which inhibit growth, but are rendered harmless by the Fungus. There is, however, also a possibility that there are certain bios substances in the agar, which are not present in peatmoss. If the latter view is correct, the state of affairs in this instance is very similar to that in the Orchids. In fact, more and more cases are being found where the influence of one partner on the other is based on the secretion of unmistakable bios substances. This is also the case in the classic example of symbiotic association, the Lichens.

In 1869 S. SCHWENDENER [596] spoke of helotism, because the cells of the Alga did not fructify and were sometimes emptied by the haustoria of the Fungus, but the true relationship of the two partners was not explained. Neither was this the case when some years later M. TREUB [657] studied the same subject. A study of quite simple cases of symbiosis in Lichens convinced A. QUISPEL [525] a few years ago that in such mutual symbiosis the exchange of bios substances is a very important factor.

Turning now to the subject of ectotrophic mycorrhiza, we must discuss that of *Monotropa hypopitys* separately. As early as 1882 KAMIENSKY [309] described this mycorrhiza in detail, pointing out that this species does not contain any chlorophyll, so that it must derive its nutrient either from the Fungus or from the organic substances in the soil. If the former is the case *Monotropa* should be termed a parasite, if the latter, a saprophyte.

Nearly 40 years later L. REXHAUSEN [540] resumed the discussion

with the assumption that raw materials assimilated by *Monotropa* were supplied by the Fungus which in turn obtained proteins from its host. This was a mere hypothesis, for no proof was available and not until 1934 were the results of an exhaustive investigation on modern lines concerning this subject published by H. L. FRANCKE [181], a pupil of BURGEFF. According to FRANCKE, the Fungus is one of the Basidiomycetae, and its mycelium with its conjugated nuclei is cited as evidence in support of this thesis. It appears that the remarkably small seeds of *Monotropa* can germinate only when the inhibiting substances, which also play an important part in the germination or non-germination of other seeds, have been washed out by leaching. Subsequently, without any fungal infection, a miniature embryo is formed in the course of one year, but then development ceases until there is infection from the special Fungus. The hyphae penetrate into the epidermal cells, forming no more than one haustorium in each cell. This grows towards the nucleus and at its top a small vesicle is formed which bursts, its content being dispersed throughout the host-cell. Thus this is a clear case of ptyophagy. The stronger the ptyophagy is, the better *Monotropa* grows, hence it is clear that this plant parasitizes on the Fungus, the latter in its turn living in symbiosis with different trees, Coniferae or Cupuliferae. In a soil with a low humus content the subterranean parts of *Monotropa* possess many ramifications, which is not so in a soil rich in humus, where it is surrounded by a dense ball of hyphae. FRANCKE is of the opinion that this is a case of mutual symbiosis, but in the view of the present writer it is questionable whether the Fungus derives anything at all from *Monotropa*, especially while the apical parts of the hyphae liberate water or diluted solutions. On the other hand the basal parts take in various substances, and these basal parts are situated not in the host plant but in the soil or in the roots of the trees.

We still have to discuss the true ectotrophic mycorrhiza where the Fungus spreads its mycelium like a cloak round the tiny roots and is connected with the environment by a great number of hyphae. This type of mycorrhiza, which occurs with Coniferae and Cupuliferae, especially with the latter, was first studied by A. B. FRANK [182] in 1887. Among more recent investigations we must mention the work of J. PEKLO [500], who observed how the hyphae penetrate into the calyptra. It was found that in many cases the Fungus was one of the Basidiomycetae, either a Hymenomyceta or a Gasteromyceta. Species of the genera *Boletus*, *Amanita* and *Cortinarius* are often encountered in such symbiosis, sometimes one species of Fungus with one particular species of tree, thus in Denmark *Cortinarius hemitrichus* is found in association with the birch.

Another investigator whose work is of importance in this connection is E. MELIN [420], who also studied symbiosis from a physiological point of view. In pure culture growth of the Fungi was usually poor, though it improved on addition of phosphatids. In view of the fact,

observed by B. HANSTEEN-CRANNER [232], that phosphatids are liberated by living vegetable cells, there was every reason to assume that such phosphatids might have an attraction for the Fungi. However, this has not been proved, nor was there any trace of nitrogen-fixation by these Fungi in pure culture.

The old experiments by NOBBE [463] showed that Cupuliferae and Coniferae could be grown with excellent results in sand without humus, and that the development of a great number of roothairs resulted, which does not occur in the presence of mycorrhiza. In these circumstances the mycorrhiza was indeed lacking. This fact led STAHL [620] to formulate his theory, referred to above, that with the aid of mycorrhiza formation the tree turns the rival Fungus into useful servant. Proofs of this thesis, however, are lacking. MELIN did find that *Pinus sylvestris*, which had been grown on sterile soil, could not make good use of complicated nitrogen compounds, whereas those with mycorrhiza did. It might be that the Basidiomycetae assimilated such products and passed them on the roots, but how? PEKLO believed he had observed that hyphae of ectotrophic mycorrhiza were digested, though this has not been seen by any other investigator. E. JAHN [293] made the entirely novel statement that the outer layer of hyphae round beechroots acted as a buffer, which he regarded as an explanation of the fact that this plant without mycorrhiza grows well in waterculture even if the p_H of this solution is between 3 and 4, whereas in natural soils the optimum p_H is between 6 and 7. If this is so, it might well be explained differently.

It might be argued that with this ectotrophic mycorrhiza the most likely view is that of mutual parasitism. If *Boletus* fructifies only when its hyphae have penetrated into *Larix europaea*, the Fungus will win, but if PEKLO is right in his statement that hyphae, which have penetrated into tissue of the host, may be digested, the reverse is the case. There is a possibility, however, that in this instance also there is a mutual supply of bios substances, for G. LINDBERG [377], for example, found that the Hymenocytes, which form mycorrhiza, generally require the bios substance aneurin. Experiments of J. EASTON HOW [279] have established that a water soluble substance capable of stimulating the growth of *Boletus elegans* is present in the roots of European and Japanese Larix but not in those of Scotch pine.

From the observations of P. M. WEST [712] it appears that the excretion of significant amounts of aneurin and biotin from young roots of higher plants occurs normally even under sterile conditions and accounts at least in part for quantitative and qualitative differences characteristic of the bacterial flora of the rhizosphere.

In any case it is obvious that all manner of transitions occur between obligatory and optional symbiosis. Ectotrophic mycorrhiza is a condition of unstable equilibrium which can be disturbed under the influence of all kinds of factors. The condition of true mutual symbi-

osis may change into mutual or one-sided parasitism, just as the harmless saprophyte may turn into a noxious parasite. "Natura non facit saltum," as was said at the beginning of this chapter.

A sign of progress in regard to this view is the renewed introduction of PFEFFER's idea of disjunctive symbiosis by W. B. MAC DOUGAL [398]. This term denotes the mutual dependence, even essentiality, of two organisms or groups of organisms living side by side in the same place. Such disjunctive symbiosis is related to ordinary symbiosis by numerous links. We need only remember the investigations by A. NIETHAMMER [461] who compared the respective development of various sorts of plants in infected and in sterilized soils.

(D) Parasites.

As already stated, mutual symbiosis may turn into parasitism, but there are also cases of a more typical parasitism. Yet, here too it is possible for the originally harmless partner to develop gradually into a noxious parasite, for instance with the smut Fungi, Ustilaginales.

It is impossible here to discuss the great number of parasitic Fungi. The subject of parasitic diseases caused by Fungi and bacteria forms an important chapter of phytopathology, a science which has developed enormously during the past fifty years. The influence of these parasitic Fungi on their hosts varies widely in nature. There are, for instance, the witches brooms, originated by the presence of an *Exoascus* which causes the sprouting of all the dormant buds in the infected spot, or the effect of *Ustilago* or *Tilletia*, causing the smut of cereals, or the effect of ergot, *Claviceps purpurea*, in rye.

The formation of spots on leaves is due to the development of rust Fungi, or Uredinales, in the leaves, whilst canker is the result of the action of the Fungus *Nectria galligena* in the stems of trees. Although most of the causes of such parasitic diseases were known in 1895, in the majority of cases the details of the course of the disease did not become known until later. But this does not imply that in such case the nature of the process itself is fully understood.

The parasites among the Phanerogams can be divided from a physiological point of view into semi-parasites, which derive only dissolved salts from their host, and complete parasites, which also obtain organic products. Between parasites and autotrophic plants there are transitional stages which will be dealt with later.

Morphologically, a division can be made into root- and stem-parasites, and more recently A. Sperlich [617] drew up a classification according to their histological structure.

A discussion on the Rhinanthaceae, which have been studied by E. J. L. HEINRICHER [246] in particular, is a suitable approach to this part of the chapter, as these comprise a practically complete series ranging

from normal autotrophic species to typical parasites. The series begins for instance with *Euphrasia odontites*, which is found in western Europe. This is a kind of eyebright with well-developed roots and roothairs, which is, in this respect indistinguishable from other autotrophic plants. Other species of this genus, for instance *Euphrasia Rostkoviana*, do germinate independently, but their development is poor until they have reached roots either of individuals of the same or of some other species. Then they form haustoria, suckers, which penetrate into the roots of their host and effect a connection with the water transport routes. One *Euphrasia* individual may thrive at the expense of others which languish.

Melampyrum arvense, cow wheat, also seems able to develop for a while without a host; other species of this genus are more dependent, their root system being poorly developed and having hardly any root hairs.

Tozzia alpina, Rhinanthacea from Central Europe, comes nearer to being a complete parasite. For germination its seed is dependent upon the presence of a host in its immediate environment. The young plant exists as a non-chlorophyllous parasite under the ground and does not appear above grond until later, when chlorophyll becomes formed and the peduncle appears. The tropical species of *Striga* again show other variations, *Striga lutea* behaves like *Tozzia alpina*, but *Striga orobanchoides* remains underground but for the peduncle and continues to exist as a parasite. *Lathraea clandestina*, a Central European species, also exists underground as a parasite on roots of trees, and only the peduncle appears above ground. The rhizome is covered with a great number of peculiarly shaped bracteae, the so-called scales, the cells of which are chock-full of starch, at least before the development of the peduncle and its flowers.

As these Rhinanthaceae do not form haustoria when there are no host-roots in the vicinity, HEINRICHER assumes that the latter exert a chemical stimulus which leads to the formation of these organs. That it is a chemical stimulus may be gathered from the observation made with *Orobanche cumana* which parasitizes the roots of sun flowers and the germination of which is initiated by an extract of these roots.

SPERLICH [617] studied the way in which haustoria penetrated as well as their anatomy. He considered that in the case of *Rhinanthus* and *Melampyrum* a connection with the xylem was effected mainly by mechanical means, whereas in germination of *Lathraea squamosa* the root of the host is surrounded by a number of haustoria, which first effect a connection with the xylem and later also with the phloem.

Further progression of parasitism is coupled with increasing reduction. The semi-parasitic Rhinanthaceae still possess normal leaves, in contradistinction to the scales of a complete parasite like *Lathraea*. This reduction is even more advanced in *Pilostyles* and *Rafflesia*, where only a kind of thallus is present, consisting of long rows of cells and situated

in the tissue of the host. In the basal part of the shoot, however, from which the flower is to be developed, the parasite forms true spiral tracheids and other vascular elements.

In regard to the green Rhinanthaceae the physiological question arises as to which is more essential to the parasite, the intake of water or that of salts. Notwithstanding the pale colouring of the species of the genus *Rhinanthus*, due mainly to the small quantity of chlorophyll in the bracteae, their process of photosynthesis has a fairly normal intensity. In these species HEINRICHER stresses an insufficient intake of salts which has to be supplemented with the aid of the haustoria, whereas KOSTYTSCHEW [344] points to an insufficient intake of water. The latter author argues that *Euphrasia* and *Rhinanthus* have an intensive transpiration, which causes discrepancy between intake and loss of water, and that this discrepancy can be remedied only by means of the haustoria. A specimen of *Rhinanthus* with haustoria evaporates up to ten times as much as a specimen of equal size without haustoria.

How does the intake of water from the host occur? In regard to this question it is obvious to assume that the parasite possesses stronger suction pressure than its host. Indeed, E. BERGDOLT [41] found that this was the case. In an instance where a species of *Orobanche*, broom-rape, parasitized the broad bean, *Vicia Faba* he found that the suction pressure in the root of the host amounted to 8 atmospheres, in the haustoria of the broom-rape it was approximately 12 and in the bracteae 19 atmospheres. In a case of a *Lathraea squamosa* (tooth wort) parasitizing *Prunus*, the suction pressure in the haustoria was approximately 32 atmospheres, that in the tissue of the host about 5. Suction pressure in other organs of *Lathraea*, however, was lower, and it is not possible to draw hard and fast conclusions from the values stated. With *Orobanche cumana* A. RICHTER [544] observed a strong liberation of water, though not so much by way of stomatal transpiration as by glandular hairs serving as active hydathodes. In this connection it should be mentioned that in the majority of cases the ash of the parasite has a quite different composition than that of the host.

On several occasions the question of the connection between the transport routes in the parasite and the host has formed a subject of research. In dodder, *Cuscuta*, this problem was studied by W. SCHUMACHER [592] with the aid of the fluorescein method, mentioned above. This parasite contains some chlorophyll, but Miss HENRICI [251] proved that its photosynthesis barely balances its respiration. SCHUMACHER also studied the anatomical structure of the haustoria of a species of *Cuscuta* on *Pelargonium zonale*. These have no true sieve tubes, only cells whose contents are rich in protoplasm and possessing excrescences which penetrate between the parenchyma and the sieve tubes of the host. In this process they dissolve primary and secondary cell-wall layers, though they spare the walls of the sieve tubes. A number

of such excrescences settle against the sieve tubes and serve as organs of resorption. When the solution of fluorescein is inside the host, it passes from the sieve tubes into the protoplasm of the excrescences. Experiments to ascertain the increase in dry weight of *Cuscuta* show that the translocation of sugars is rapid and has a rate of a few cm. per hour, which again raises the question of the cause thereof. Virus also passes from the host to the parasite and vice versa.

Viscum album, mistletoe, repeatedly formed a subject of research during the present century. Here the only connection is between the xylem of the host and the parasite, for ringing wounds made in the branch of the host below the place where the mistletoe is implanted do not interfere with the intake of nutrient by the parasite. The branch, however, does not grow very far distally beyond the point of attachment, hence it may be concluded that the mistletoe draws a strong stream of water and salts towards itself. According to that the suction pressure of the parasite must be considerable. Many parasites on one host, cause its death. Examination of the ash-content of *Viscum album* showed it to differ considerably from that of its host, both quantitatively and qualitatively, which certainly does not suggest the existence of an open connection between the xylem vessels of the two.

As was shown by investigations of J. WIESNER [727] as early as 1884, the pulp of *Viscum album* contains substances which have an inhibitory effect upon the germination of all kinds of seeds, even upon the mistletoe's germination When these substances are removed, germination is still only possible on the branches of the species functioning as host. According to some investigators this germination is accompanied by a secretion of substances, particularly of an enzyme xylase with wood-dissolving properties, which attacks the woody tissues of the host. However this is probably not correct. The root does not really penetrate into the wood already formed, for the tip of the sucker is situated in the place where the cambium of the host-branch was at the time of infection and the growth in length of the sucker keeps pace with the growth in thickness of the host branch.

The question as to what species of trees are parasitized by mistletoe is of importance also from a physiological point of view. A survey of the entire West-European region where mistletoe occurs shows that these species are about 30 in number, but it is noticeable that the mistletoe has a distinct preference for a few species, particularly in the northern part of this region as was shown by an investigation of LAURENT [366]. In the Netherlands, mistletoe occurs practically exclusively in the southern part of the province of Limburg where the soil contains lime. The mistletoe is here confined to the Canadian poplar, Populus canadensis serotina and the apple tree. It would lead us too far to enter into the question as to whether there are different strains of *Viscum*.

HEINRICHER [246] showed that different species and varieties behave in entirely different ways towards *Viscum album*. This is especially

noticeable in different varieties of pear trees. Some varieties behave like the apple and do not react to the infection, others are completely immune and the seeds of the mistletoe do not germinate, and finally, there is a category where infection results in constant struggle and the host tries to ward it off by the formation of crustlike tissue. It is not possible to enter fully into a question which is interesting from a plant-geographical point of view, namely if the fact that mistletoe is found especially on host-plants rooted in lime-soil has any connection with their greater or lesser susceptibility to the infection.

(E) Insectivorous Plants.

Of the heterotrophic plants only the insectivorous plants remain to be dealt with. Their photosynthesis is similar to that of autotrophic plants, though in addition to the intake of inorganic salts by means of the roots they obtain nutrient elements from their victims.

Only in one instance, *Utricularia*, need the way in which the trapping is done be described in greater detail. The main points were sufficiently well known at the end of the past century and apart from that one instance our conceptions of it have not been modified to any great extent. The processes of motion, which play a part in the process of trapping, will be dealt with later in the discussion on the movements of plants, but in this connection chemotropism and chemonastic play the chief parts.

What needs to be considered here is the way in which the prey is digested and its usefulness to the plant. During the past century hardly any research was made in this respect, apart from the investigations of CHARLES DARWIN [132] and his son, FRANCIS DARWIN [133]. The secretion of enzymes was likewise studied only sporadically, for instance by J. D. HOOKER [271] in *Nepenthes*.

In the discussion of these matters it should be stressed that insectivorous plants exist in an environment where, generally speaking, mineral components are but scarce. They are found on peatmoors, where the substrate is a *Sphagnum* vegetation, which is poor in minerals. Other insectivorous plants such as the *Utricullariae* are a characteristic feature of the flora of oligotrophic waters, i.e. waters poor in nutrient.

It is obvious that the trapping of insects must be useful to the insectivorous plants, although this by no means implies that it is essential. Despite G. SCHMID's [578] assertion, the chlorophyll content of insectivorous plants cannot be regarded as specially low, so that there is no reason to assume that the trapping of insects is important in view of the acquisition of carbon compounds. H. WEYLAND [717] drew attention to the fact that the roots of insectivorous plants are poorly developed and that an analysis of them reveals little potassium or phosphoric salts. Compare this with SCHMID's observation that in the tentacles of *Drosera* before the trapping of insects no phosphate or potassium ions occur, though a great many are present afterwards, and it will be clear in what way

the trapping of insects is most useful and that it is by no means only a matter of obtaining nitrogen.

But these were only casual observations. The problem, however, was studied in the laboratory at Groningen, first by J. OOSTERHUIS [476] and later by J. OUDMAN [485]. The former confined himself to the question as to whether by the trapping of insects (green fly) a deficiency of nutrient salts in the soil was met. He grew seedlings and winter buds of *Drosera intermedia* in a nutrient solution, taking the dry weight as a measure of development. He concluded that plants grown in a nutrient solution from which either nitrogen, potassium, phosphor or magnesium was absent remained small and that such was also the case if they were grown in a complete nutrient solution, the so-called Knop's solution. However, if sufficient supply of green flies was made available, the plants developed satisfactorily when grown in such a defective solution and even in distilled water. This led OOSTERHUIS to assume that there must be special advantages attached to the trapping of insects but in view of the following it is questionable whether, perhaps, a different nutrient solution would have yielded better results.

OUDMAN, who worked with *Drosera capensis*, did obtain good results with cultures in a Knop's solution. Growth was good whether sodium nitrate was taken up by the roots and non-nitrogenous substances by the leaves of the plants, or asparagine by the leaves and non-nitrogenous substances by the roots.

Apart from photosynthesis, both leaves and roots are capable of taking up any nutrients and they can supplement each other or take over each other's functions. The primary importance of the trapping of insects to species which have their existence on a soil poor in minerals thus becomes quite clear. In regard to the uptake by the leaves, it was observed that both the tentacles and the dorsal side of the leaf function in the intake, though the former play the chief part.

There is no need to go into aggregation phenomena occurring in the tentacles during the intake of food. These phenomena were known in the past century and were studied by DE VRIES. Though mention must be made of the fact that experiments by OUDMAN showed that narcosis inhibits the intake of salts by the tentacles as well as that by the roots. Investigations in the laboratory at Groningen revealed a translocation of asparagine from cell to cell, the intake into the cell occurring at the expense of energy obtained in respiration.

An important point in regard to the metabolism of insectivorous plants is the question as to whether they always secrete the same enzymes to digest their victim and, if so, which enzymes are secreted.

At the end of the past century the general view was that there were genera of greater and lesser specialisation. It was thought that in the latter, such as *Sarracenia* and *Utricularia*, no special enzymes for the breakdown of the proteins of the prey were secreted and that putrefactive bacteria were responsible for the digestion of the prey, whereas

in the former, such as *Nepenthes*, an organic acid as well as a proteolytic enzyme were secreted and digestion was actively carried out.

In the present century this question was studied first in tropical pitcher-plants, *Nepenthes*, in Java. During his visit to Buitenzorg, G. CLAUTRIAU [118] carried out investigations on this subject and found a slimy neutral liquid even in young pitchers, and it was not until after chemical stimulation that a secretion product with acid reaction and possessing a strong proteolytic action arose. This investigator was of the opinion that the breakdown of protein did not go beyond peptone, but S. H. VINES [680] found that the enzyme erepsin was also present, which, with a weakly acid or neutral reaction, broke down the peptones further into amino acids.

In 1932 K. G. STERN [628] and E. STERN [626] arrived at the conclusion that in the pitchers of *Nepenthes* two real proteinases were present, one being a catheptic enzyme with an optimum at the iso-electric point of the proteins (p_H 4-5), the other a tryptic anzyme with an optimum at a p_H of 8. They obtained this result both in the liquid from the pitchers and in an extract from the tissue. Particularly in view of the fact that at p_H 8 the tissue-extract obtained by means of acetic acid-glycerin has a tryptic effect on gelatine, they concluded that there was here no question of any bacterial action.

Several investigators, however, doubted this conclusion, and in 1934 JETSKE DE ZEEUW [751] published the results of some experiments made in the laboratory at Leyden, which confirmed this doubt, and it was concluded that the preparations used by the STERN's had not been free of bacteria.

When young, unopened pitchers are opened in a sterile way, only the presence of a catheptic enzyme, which becomes active at a p_H of 4-5, can be observed. After a chemical, though not after a mechanical stimulus, the glandular cells secrete an acid which causes this degree of acidity to arise. It is still unknown what this acid is.

Other pitcher-plants, like *Sarracenia*, behave in approximately the same way, as was shown by the experiments of J. S. HEPBURN [252] and his collaborators, though it seems that *Darlingtonia* produces no enzyme.

In *Dionaea muscipula*, the leaf of which does not close unless the first mechanical stimulus is followed by another within a few seconds, the glands secrete only when by capture of the prey its chemical products are able to apply a chemical stimulus. Its secretion product contains a proteolytic enzyme and an organic acid, which fact was already known to PFEFFER [504].

Drosera and *Pinguicula* secrete a slimy substance which slime causes small insects to adhere by its tackiness. Subsequently, the tentacle touched by the insect sends out a stimulus to the surrounding tentacles which bend over to the one that was touched and meanwhile undergo cytological changes. Aggregation sets in, e.i. the large vacuole is

split up into smaller ones, the cells at the tip of the tentacle beginning to show the characteristics of glandular cells. They secrete a liquid with an acid reaction and containing a catheptic enzyme. In the case of *Drosera* the nature of this acid is still unknown, but according to LOEW [388] and ASO [18] in the butterwort, *Pinguicula vulgaris*, benzoic acid is present.

In regard to *Utricularia*, the general view is that in this case no enzymes are secreted. In 1910 PH. VON LÜTZELBURG [391] argued a contrary opinion, but his arguments were not very convincing.

There has been a great deal of controversy on the mechanism of the opening and closing of the vesicles of bladder-wort, *Utricularia*. Investigators of the previous century, such as DARWIN considered this was something like an insect-trap,the structure of which could be compared to that of a fish-trap. In the present century F. E. LLOYD [385] drew a completely different picture of the matter, as a result of close observation with the aid of film-microphotography. LLOYD compared the closure of the small bladder to a door with a strong rabbet, the door only opens inward, but is pressed against the rabbet by the tension of the tissue. When it has been closed for some time, the pressure in the bladder will fall, because the tissue absorbs gases whilst the tension of the tissue prevents water from entering along the rabbet. When a victim comes near and touches the hairs by the door, the vesicle will change slightly in shape, so that the aperture is no longer closed. Suddenly water streams in carrying the victim with it, but within 1/16th part of a second the vesicle resumes its former shape and the door is closed again. By being pressed against the rabbet the door once more closes the bladder hermetically.

CHAPTER VIII

GROWTH (ELONGATION) AND PHOTOTROPISM

(A) Introduction: Growth.

Again we start from the stage reached by plant physiology in 1895. At that time growth and the movement of plants were dealt with as two entirely separate entities, though it will be seen presently that a strict separation between the two is hardly possible, particulary in a historical discussion of the subject.

In HUGO DE VRIES's observations concerning growth the chief part is played by turgor. According to this author, it is turgor which determines the rate of growth, notwithstanding the obvious fact that the nature of the cellwall is also an important factor.

The old controversy regarding the growth of the cell-wall itself, whether this was due to apposition, the formation of new layers up against the old ones, or to intussusception, the interposition of the new particles between the existing ones, was solved. By means of a staining process F. NOLL [465] demonstrated apposition in the cell-walls of *Caulerpa* whereas the occurrence of intussusception in root hairs was proved by E. ZACHARIAS [749]. The conclusion must be that both processes occurred.

The question as to whether the cell-wall underwent a plastic or an elastic stretching or whether both processes occurred, remained undecided and no more was said about the matter than that the living protoplast must be the cause of the changes in the cell-wall.

The movements of cells and organs were viewed as the result of external stimuli, at least the majority of them. For example, the stimuli of gravity and light were supposed to cause unicellular organs such as hyphae to curve, owing to the fact that the elasticity of the wall had become greatest on the side which was later to become convex. In multicellular organs the growth in length of the cells on one side of the organ was said to be greater than the growth on the other side and so to cause curvature. As stated, DE VRIES considers that turgor plays the chief part in this growth.

It was known that parts of plants generally grow more rapidly and more intensively in the dark than in light, but DE VRIES stated explicitly

that the curvature, which was then termed heliotropic, not phototropic, was by no means a result of the fact that under lateral illumination one side received more light than the opposite side which is turned away from the light. For if that were the case, he said all organs would be positively heliotropic, whereas in roots a negative heliotropism i.e. a turning away from the source of light, is observed.

What causes curvature to arise from the stimulus of gravity, or the question why the roots have a positive geotropism and the stems a negative geotropism, was still entirely obscure.

It was known that sensitivity to the stimulus of gravity is seated in the utmost tip of the root, but not in the actual region of curvature. This was clearly shown by the work of Ch. Darwin [132] of 1881, and it might be concluded that the stimulus must be conducted from the tip of the root to the zone of curvature. In some cases of light stimuli it was also considered that conduction of stimuli occurred, for instance in seedlings of some Gramineae studied by W. ROTHERT [554].

(B) Study of Phototropism up to 1927.

At the beginning of the present century the study of phototropism came entirely under the influence of the observations in PFEFFER's "Auslösungstheorie" which contains many elements derived from animal physiology.

It was considered that the living protoplasm was in a condition of metastable equilibrium and passed into another, likewise unstable, condition as a result of stimulation. Through perception of the stimulus a quantity of potential energy, which was accumulated in the plant, was set free, though this quantity was by no means proportional to the energy required for the stimulus itself. In this connection was used the well-known comparison of the energy required for pulling the trigger of a gun with the energy set free by the shot. Nor was there any proportionality between the intensity of the stimulus and the reaction, although the former had to exceed a certain minimum or threshold value.

The degree of reaction depended not only on the perception but also on the connection between the perceiving organ and the part carrying out the reaction. PFEFFER spoke of a stimulation - chain; the tonus — expressed by the type of reaction — resulting from the condition of the protoplasm concerned. The time elapsed during perception, conduction and preparation of the reaction, up to its commencement, was termed the latent period or theoretical reaction period. As we shall see later, there has been a good deal of discussion concerning the idea of a present-ation-time, which is the shortest period for a reaction to be initiated.

PFEFFER's theory led to a search for the sensitive organs of plants, particulary to the investigations of G. HABERLANDT [222], whose work entitled "Die Lichtsinnesorgane der Laubblätter" (the light-sensitive organs of the leaves) appeared in 1905. At this juncture it is not possible

to enter into details as regards these sensitive organs. HABERLANDT distinguished light-sensitive organs for the perception of light in the leaves, statoliths for response to gravity in root -cap and the starch-sheath, whilst in the epidermis of tendrils he found tactile organs. Despite the labour and acumen lavished on this subject, the function of the structures concerned was not proved conclusively and we shall see later that in the light of the auxin theory the existence of such sensitive organs needs not be discussed.

Investigations published by H. FITTING [144] in 1907 shed new light on the conduction of the phototropic stimulus in the coleoptiles of the Gramineae. It was found that this stimulus is transmitted laterally as well as lengthwise and is not impeded by two incisions on opposite sides of the coleoptile up to 3/4 of the width. But heating of a section to 40° C does inhibit conduction, whilst the lethal temperature lies at 43° C.

A few years later P. BOYSEN JENSEN [69] gave a different view of the matter, when he established that there was a conduction of the stimulus from apex to base if both parts were separated from each other by a cross cut and connected together again by means of gelatine. It followed that in the conduction of a stimulus there must be translocation of some substance, a kind of diffusion right through the gelatine. BOYSEN JENSEN summarized his conclusions thus: "under the influence of one-sided illumination polarity is set up in the apex of the coleoptile, such polarity being due to the uneven distribution of some substance at the front and the back. At the back this substance is translocated to the base, where it causes acceleration in growth, which in turn causes phototropic curvature."

His work led to the tests made by ARPAD PAAL [490] in 1914. This investigator caused a stimulus to be conducted through thin cross-sections of the stem of *Calamus Rotan*, placed between the apex and base of the coleoptile and soaked in gelatine. To prove that not any effect was due to electric currents a thin sheet of platinum was placed between apex and base, whereupon there was no further conduction of the stimulus. ARPAD PAAL also obtained curvatures when he placed the tip of seedling which had been subjected to one-sided illumination on the base of another decapitated seedling of the same species which had not been illuminated. Hence conduction must have occurred by the diffusion of a substance in solution, which was produced in the tip.

Up till then all this had been viewed only as a detail of the subject of phototropism, but a few years afterwards ARPAD PAAL for the first time associated this process of stimulation with the phenomena of normal growth-regulation of an unstimulated plant. He regarded the tip as the centre of growth-regulation and considered that in the tip some substance or a mixture of substances was constantly being produced, which in unstimulated parts is evenly distributed along all sides of the basis and causes growth in the zone of elongation. Under one-sided illumination this substance was unevenly translocated to the base,

mostly on the side not receiving illumination, and would, therefore, cause strong growth on that side. This meant that growth in length and phototropism are connected; in the opinion of PAAL there are no special wound -or stimulus substances, but the concentration of the substance required for all growth is unequal.

However, before continuing this trend of thought, an entirely different series of investigations must be considered.

The work by F. OLTMANNS [473] in 1897 showed that in *Phycomyces nitens* positive as well as negative phototropism could occur: strong light caused a negative, and weak light caused positive phototropism. At intermediate light-intensity no response occurred at all. In describing these tests OLTMANNS used a term borrowed from the study of geotropism, viz. presentation-time, to denote the shortest period of stimulation which would bring about a reaction.

A. H. BLAAUW [51] realized that all this was very vague and that more thorough research and clearer definitions were required. He began his own investigations by ascertaining the threshold value and presentation-time in coleoptiles of *Avena sativa*, a plant which was to become the favorite test object for the study of phototropism and the auxin-theory. BLAAUW soon saw that, contrary to the view of former investigators, there is no presentation-time in phototropism, nor any particular intensity of light required for the stimulus. The response is governed by the product of the length of time and the intensity of the illumination, the number of meter-candle-seconds which phenomenon is termed by BLAAUW the "product rule". This is shown by studying the so-called threshold value of the reaction, which is defined as the quantity of light-energy which in about 50% of the specimens causes a curvature just visible with the naked eye.

With a light-intensity of 0.05 M-candles the time required is approx. 8 min., product 24 M-cdle.seconds.

With a light-intensity of 0.1 M-candles the time required is approx. 4 min., product 24 M-cdle.seconds.

With a light-intensity of 1 M-candles the time required is 25 seconds. product 25 M-cdle seconds.

Thus the quantity of light, or light-energy, is the determining factor. Tests made with *Phycomyces nitens* confirmed this theory, which was already stated by NATHANSOHN [450] and by E. PRINGSHEIM [521] after experiments with intermittent illumination.

Curiously enough there appeared almost simultaneously with BLAAUW's work a publication describing a similar investigation by P. FRÖSCHEL [188] with *Lepidium sativum*, which led the latter likewise to formulate a product-rule.

BLAAUW pointed out, moreover, that what was observed in this regard in plant physiology also held good in a photochemical reaction. OSTWALD [488] had stated: the photochemical effect equals the product of time and intensity. This similarity caused BLAAUW to ascertain which region of

the spectrum causes the strongest effect in phototropism. His result was an optimum in the blue region at 466–478 λ for *Avena* and at 495 λ for *Phycomyces*. In the green region sensitivity was very slight, and in the ultra-violet it was only 25% of that in the optimum.

The negative reaction of Phycomyces, which requires a great number of meter-candle seconds, was also subjected to closer study by BLAAUW and compared with the solarisation of a photographic plate, caused by over-exposure. BLAAUW was inclined to view the entire phototropic question as the resultant of two opposite processes. This was similar to NERNST's [455] view that a substance which is sensitive to light is a system in which two opposite reactions occur simultaneously.

A plant grown in the dark has a peculiar sensitivity to light, which is much greater that that of a specimen of the same species grown in light. In the latter the threshold value lies at a much higher level. F. OLTMANNS [473] and E. PRINGSHEIM [521] used the term "tonus" to denote this. A non-living system which is sensitive to light also has a photo-chemical equilibrium and the phenomena of over-exposure in a plant and in a photochemical plate run parallel. BLAAUW therefore considers that this sensitivity in the plant must be due to the presence of a system sensitive to light.

Not long after BLAAUW's thesis was written in the laboratory of F. A. F. C. WENT [709] there appeared between 1914 and 1918 three publications by BLAAUW on "Licht und Wachstum" (Light and Growth), the' first of which dealt with investigations on *Phycomyces nitens*. BLAAUW was convinced that in order to gain an insight in phototropism, it was necessary first to study the response of the living plant-cell to all sided illumination. For this a test had to be arranged in which growth was measured far more accurately than had been possible before. A requisite was that the temperature should be kept constant within approximately 0.1° C, as the test plant reacted to very slight rises in temperature.

The result of these tests was that *Phycomyces nitens* was found to have a clearly discernible increase in growth after 3 to 4 minutes, when a certain quantity of light-energy, for instance of 120 meter-candle seconds, was evenly administered from 4 sides. BLAAUW named this reaction the lightgrowth response. The phototropism occurring after one sided illumination he explained from the lightgrowth response of the front and the back of the sporangophore by arguing that this might be regarded as a transparent cylinder in which the rays are concentrated at the back.

If large quantities of light-energy are administered from 4 sides, the course of events becomes more complicated, as in that case the positive lightgrowth response with acceleration of growth is followed by a decrease of growth after approximately 25 minutes. In this case it also becomes difficult to avoid a change in temperature.

In connection with the study of phototropism in *Avena sativa*, it was

considered desirable also to ascertain the lightgrowth response in multicellular objects. For this investigation BLAAUW selected the seedling of *Helianthus globosus*, the hypocotyl of which shows positive phototropism after one-sided illumination. It was found that in this instance the lightgrowth response was negative. When the seedling was grown in the dark, four sided illumination with 32 meter-candle seconds caused a clearly discernible decrease in growth. BLAAUW argued that in this object the intensity decreased as a result of light-absorption in the non-transparent tissue and the back receives less light than the front. Thus the negative lightgrowth response is stronger in the front and one sided illumination must result in a curvature towards the source of light. BLAAUW stressed in particular that phototropism is a secondary, growth the primary phenomenon. The angle at which the light rays enter, is in his opinion not important, but according to BLAAUW it is the difference between lightgrowth response, varying in strength on both sides, which causes curvature.

BLAAUW's views are more or less a modern version of the old theory of A. F. DE CANDOLLE [105] of 1832. But what happens in those parts of the plant where there is negative phototropism? For it was on the strength of these particular test objects that the theory of DE CANDOLLE was rejected in SACHS's days. Negative phototropism was especially marked in roots and these were, therefore, included in BLAAUW's investigations. He soon found that various roots, for instance those of *Lepidium sativum* and *Avena sativa*, showed hardly any lightgrowth response and had no phototropism. The roots of *Sinapis alba* do have a lightgrowth response, for they react negatively under strong 4 sided illumination, and phototropic curvature may also be observed. This is undoubtedly an argument for the connection between lightgrowth response and phototropism, assumed by BLAAUW, but the problem still remained, how to explain why this negative phototropism should arise from the negative lightgrowth response. BLAAUW revived the idea of WOLKOFF [740] and HOFMEISTER [265] and argued that there is only little light-absorption in the transparent root tissue, whilst the curved surface causes refraction of light, as a result of which the intensity of light is stronger at the back of the root than at the front. This must be the case particularly in the utmost tip of the root, which was known to be the place where geoperception takes place. The stronger illumination of the back side results in a stronger negative lightgrowth response, in other words the back grows less than the front and the curvature is negative.

Summarizing his investigations extending over four years, BLAAUW concluded that the problem of phototropism has, instead, become the problem of unequal lightgrowth response at back and front. According to him it is the lightgrowth response itself which requires explanation, for instance the contrast between the positive lightgrowth response of *Phycomyces nitens* and the negative response of the other objects studied.

These three publications by BLAAUW form one whole and we should

try to ascertain to what extent his theories agree with the ideas put forward by contemporary investigators.

The thesis of 1909 was written in the laboratory of F. A. F. C. WENT[709] at Utrecht, and a number of other publications, mostly originating from that laboratory, had a bearing on it. In this context we must confine ourselves to an enumeration of the subjects dealt with and a brief description of some of these writings.

1910. C. J. RUTTEN-PEKELHARING[562]: Investigations concerning the perception of the stimulus of gravity.
1914. MA. S. DE VRIES[688]: Influence of temperature on phototropism.
1915. W. H. ARISZ[13]: Investigations concerning phototropism.
1917. U. P. VAN AMEIJDEN[7]: Geotropism and phototropism in the absence of oxygen.
1918. C. E. B. BREMEKAMP[73]: The theory of phototropism.
1920. H. L. VAN DE SANDE BAKHUIJZEN[566]: Analysis of phototropic tonus-phenomena.
1922. V. J. KONINGSBERGER[343]: Tropism and Growth.

It is worth considering how this impressive series of publications compares with BLAAUW's work and what was the general consensus of opinions on his views.

The first two of these Utrecht theses to a great extent endorse the viewpoint of BLAAUW's thesis; the work of Mrs. RUTTEN PEKELHARING strongly argues the validity of the product rule in the perception of gravity.

ARISZ's thesis is in part a continuation of the work of BLAAUW of 1909. According to ARISZ, the degree of curvature is a function of the amount of light-energy applied. With the aid of the microscope it is possible to observe extremely small curvatures down to light energy of only 1–2 M-candle seconds. BLAAUW using the same experimental plant, *Avena sativa*, and observing with the naked eye, found the threshold value at approximately 20 M-candle seconds.

The tests by ARISZ revealed that up to about 100 M-candle seconds the increase in curvature was proportional to that of the quantity of light. When that quantity was increased still further the proportionality did not hold good any more; the curvature decreased and at about 4000 M-candle seconds turned into a negative reaction. A considerable part of the thesis is devoted to careful analysis of the tonus-phenomena. ARISZ concludes that the reaction arising after first illuminating a coleoptile from all sides and subsequently from one side only, is the result of the total quantity of light-energy falling on the coleoptile.

The thesis by KONINGSBERGER gave a description of a new self-registering auxanometer, enabling measurements of growth to be carried out in complete darkness and on the clinostat. One of the various results obtained by this apparatus was that the retardation of growth caused by a particular quantity of light-energy was proportional to the sensitivity calculated from the degree of curvature. This fits in with BLAAUW's trend of thought.

Before 1920 there was a strong inclination on the part of botanists of other countries and especially in Germany, to reject BLAAUW's later views, as they only constituted a more up to date version of the old theory of DE CANDOLLE of 1832, which had been completely rejected by SACHS. Moreover, the views of PFEFFER and FITTING [178] regarding the "Auslösungstheorie" could not be brought into line with BLAAUW's theory.

After 1920 the tide turned. In 1923 L. JOST [305] stated in the last edition of his "Vorlesungen," edited by him in collaboration with BENECKE [38] under the title of „Pflanzenphysiologie": „Es ist nicht zu leugnen dass die BLAAUW'sche Theorie in den letzten Jahren erheblich an Boden gewonnen hat. Wir haben sie dementsprechend mehr in den Vordergrund gestellt; müssen aber zum Schlusse sagen, das noch viele Bedenken bestehen und noch viel zu tun übrig bleibt um sie zu beweisen" (It cannot be denied, BLAAUW's theory has of late years gained ground considerably. We have accordingly brought it into prominence; nevertheless we are bound to say in conclusion that there are still many objections, and that much remains to be done before it can be considered well-established.)

What were the difficulties that had to be surmounted? It stands to reason that the possibility had to be demonstrated of explaining the phototropic curvature even in the classic object, the coleoptile of *Avena*, by means of BLAAUW's theory.

It was not sufficient to argue generally that this was an object which was but little transparent to light and of which the back showed a weaker negative lightgrowth response than the front, so that a positive phototropic curvature arose. The question also had to be studied quantitatively. It was necessary to ascertain how strong the illumination of both front and back of the *Avena*-coleoptile really is and how strong the lightgrowth response will be with the actually occurring light intensities on both sides. The difference between these two lightgrowth responses would then have to result in a degree of curvature which agreed with the phototropic curvature observed in the experiments. In short, actual facts were wanted.

First an investigation by A. PISEK [510] was published. This author found no such agreement. Later, in 1927, C. VAN DILLEWIJN [138], who worked in the laboratory at Utrecht, came to the opposite conclusion. Under one-sided illumination the ratio of light-intensity between the front and the back of the coleoptile was approximately 30 : 1. When VAN DILLEWIJN determined the lightgrowth-response at 2400 × 90 M-candle seconds and at 80 × 90 M-candle seconds and calculated the curvature which should result from such growth at two opposite sides of the coleoptile, this curvature agreed with the actual observations made with one sided illumination of 2400 M-candles during 90 seconds.

Hence it seems as though VAN DILLEWIJN's work completely confirmed BLAAUW's theory, but on closer examination this is not found to be the

case. For the coleoptiles of *Avena* belong to the kind of objects where the place of perception does not necessarily coincide with that of the reaction, as was shown earlier by the investigations of ROTHERT [554]. Careful examinations showed that with small quantities of light-energy the reaction in the zone of curvature at approximately 5-7 mm. from the tip is the result of illumination of the upper 2 mm. With a greater quantity of light a so-called basal reaction also occurs in consequence of illumination of the base, such reaction setting in after about 20 minutes, whilst the apex reaction requires about an hour to set in. So there must be a conduction of stimulus in this object, but this does not fit in with BLAAUW's view. For this author strongly emphasized the comparison with a photochemical equilibrium in each cell. If, however, the linking-up of phototropism with the lightgrowth response at front and back is regarded as the main point of BLAAUW's theory, this is not overthrown thereby.

Let us now leave this question for a moment and return to the work of ARPAD PAAL [490] on the conduction of phototropic stimuli. It will be remembered that this author was of the opinion that in the coleoptile tip a substance is formed which in unstimulated parts is distributed evenly along the sides and causes growth in the zone of elongation. After one-sided illumination this substance would be translocated most on the side that was not being illuminated and cause most growth on that side.

So far, experiments had been made only with coleoptiles under one-sided illumination, but H. SÖDING [615] succeeded in establishing that the average growth of decapitated coleoptiles, which were covered with wax, after 5 hours amounted to only half of that of decapitated coleoptiles, the cut tips of which had been replaced. That the substance referred to, which was supposed to influence elongation, was not specific, was concluded by F. STARK [622] from the fact that it was possible to place the tips of coleoptiles of one species of cereal on the decapitated base of another species, without prejudice to the acceleration of growth.

(C) Auxins and their Significance in regard to Growth and Phototropism.

The decisive step, isolation of the substance, called auxin, was accomplished by F. W. WENT [710] in 1926. When 1-2mm. long tips of *Avena*-coleoptiles are cut off and placed upon agar, the auxin diffuses out of these tips into the agar, as was found when small cubes containing this agar were placed upon decapited coleoptiles. For when such agar-cubes containing auxin are placed on one side of the cut surface, the stump curves towards the side turned away from the agar-cube, and the curvation may be used as a measure for the quantity of auxin in the agar.

It is found that, within certain limits, growth is proportional to the quantity of auxin administered. Without auxin there is, generally speaking, no growth. WENT based his further observations on the

assumption that the quantity of auxin and material for cell-extension are the only limiting factors in the growth of oat-coleoptiles. In these the auxin is translocated from the tip to the zone of elongation, hence in a basal direction. But this translocation is strongly influenced by illumination. For instance, light of 1000 M-candle seconds radiated all round causes a decrease in the quantity of auxin which diffuses from the tip to the zone of elongation. WENT views this decrease as the cause of the negative lightgrowth response.

If the coleoptile is illuminated from one side only, the stream of auxin, which would otherwise diffuse evenly towards the base, is diverted in such a manner that the side on which the light falls receives much less auxin, the shaded side receiving excess. This difference in the auxin supply WENT regards as sufficient explanation of the phototropic curvature.

At first WENT was of the opinion that the discovery of auxin and its action would fit in with BLAAUW's idea, but when he became convinced that phototropic curvatures cannot be explained by the lightgrowth response of the light-and shaded sides, he had to renounce that opinion. He regards phototropic curvature as the combined effect of decrease in auxin production through light and shade and of diversion of auxin.

In his thesis WENT gave a few data regarding auxin itself. He found it to be a substance which resists heating to 100°C, possesses a molecular weight of approximately 350 and specifically increases the extensibility of the cell-wall.

The problem now became one for the analytical chemist! The quantity of auxin which could be obtained from the growing points of oat-coleoptiles was so infinitesimal that further chemical examination of it was an impossibility. With some collaborators F. KÖGL [339] prepared some auxin from 100,000 maizecoleoptiles, but even that quantity was so small that it was possible only to establish its acid character. Therefore KÖGL's idea to try and isolate auxin out of human urine proved to be a fruitful one.

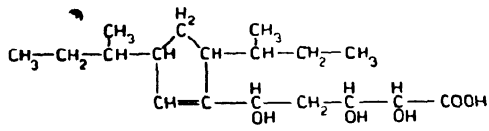


FIG. XXI. Auxin a

After a most difficult and lengthy chemical research, which cannot be gone into here, it was found to be a crystalline substance which KÖGL termed auxin-a, $C_{18}H_{32}O_5$, molecular weight 328.

How very little of it is present in one coleoptile is shown by the fact that one Avena-unit, or the quantity of auxin, which under certain conditions of temperature and moisture produces a curvature of 10° in 90 minutes, amounts to only $\frac{1}{50 \times 10^6}$ mg.

Other auxins were also obtained. Out of maize oil KÖGL isolated auxin b, formula $C_{18}H_{30}O_4$.

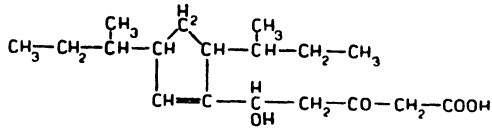


FIG. XXII. Auxin b

In a weakly acid solution the auxin a is in equilibrium with the auxin a lactone.

From the mould culture of *Rhizopus suinus* N. NIELSEN [459] obtained an auxin which he termed rhizopine and of which the chemical identtity was ascertained by THIMANN [649]. It was found that this substance,

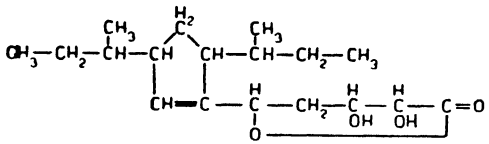


FIG. XXIII. Auxin a lactone

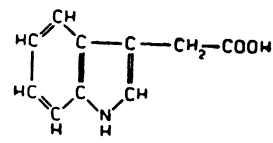


FIG. XXIV. Hetero-auxin

which has since been referred to as hetero-auxin, also occurs in human urine. The chemical structure is totally different from that of auxin a, b and auxin a lactone, being indole-acetic acid, $C_{10}H_9O_2N$.

This hetero-auxin is only half as active as auxin-a, for 1 mg. is aequivalent with 25 million Avena-units. It also differs in reaction to light, as will be seen later.

Since then other auxins have been isolated, the structure of which is more or less similar to that of indole-acetic acid, but which vary slightly in their action. HAAGEN SMIT et al. [218] isolated indole acetic acid out of a higher plant, *Zea Mays*, and P. LARSEN [364] obtained indole-acetaldehyde from *Phaseolus*.

What is the action of these auxins and how are they transported? These questions and the problem of how they arise, which is still entirely unknown, have so far constituted the chief objects of research. We shall see, in fact, that the action of auxins covers a much wider field of botany.

No doubt their action in the process of growth will be due to their influence direct or indirect, on the processes of extension of the cell-wall. Contrary to the view held by HUGO DE VRIES that turgor is the determining factor in this extension, F. W. WENT in his thesis suggested that the auxin might enhance the extensibility of the cell-wall to such an extent that it becomes plastically stretched by the osmotic pressure of the cell-sap.

In 1920 H. ZIEGENSPECK [754] found that in young cell-walls there is

a substance of great plasticity, which he termed amyloid. This gave rise to the special investigations by F. OVERBECK [486] in 1926 concerning the plasticity of growing cells. He demonstrated that when sections of the elongation zone of roots are plasmolysed, the cells contract less when they have been previously saturated with water than if they have not been thus saturated, for the enhanced extension caused by the intake of water brings about an irreversible plastic stretching. In view of the results obtained by URSPRUNG [667] and BLUM [53], who found that in the zone of elongation the elastic resistance of the cell-walls is at its minimum, WENT was inclined to regard the action of the auxins as the cause of the enhanced plasticity of the wall.

Attempts to prove this theory experimentally were made by H. SÖDING [615] and by A. N. J. HEIJN [256]. Both worked with plasmolysed oat-coleoptiles. Whereas the former made little distinction between plastic and elastic stretching, the latter regarded the change in plasticity as the primary phenomenon. If he prevented growth by cutting off the coleoptiles and leaving them without water, the plasticity of the cell-wall, though not its elasticity, was affected by decapitation or by the artificial supply of auxin. HEYN proved furthermore by bending tests that this plasticity is enhanced by the addition of auxin to decapitated coleoptiles.

No doubt an objection to these investigations is that, though a great deal is said about plasticity of the cell-wall, the nature of wall-structure is not clearly explained. This lacuna has been largely filled during the past few decades as a result of modern research in colloid chemistry and physical optics, which has taught us a great deal concerning the micellary and molecular structure of the substances of the cell-wall. For example A. FREY-WYSSLING [186] stressed the presence of sub-microscopical cellulose bricks in this wall. The existence of cellulose micelles was known since NÄGELI [449]. Cellulose itself is anisotropic, but to show that it exists as micelles FREY-WYSSLING removed the cellulose from the cell-walls and found that they were isotropic when impregnated with a solution of the same refractive index as the intermicellary material, but became anisotropic in media of lower or higher refractive index. This structural birefringence indicates the existence of micelles. They lie parallel to the wall, though at a larger of smaller angle to the direction in which the elongation of the cell will take place. Cells which may become greatly extended have transverse micellae in a so-called tubiform pattern. According to J. BONNER [59] these micellae are placed less regularly in young cells, where they comprise only 42% of the volume of the cell-wall, the remainder consisting of intermicellary substance, pectin and hemicellulose. According to THIMANN and BONNER young Avena cell-walls contain 10% pectin, whilst much of the non-cellulose constituents are hemicelluloses. With mechanical elongation these micellae assume a more longitudinal position, as may be observed with the aid of the polarization

microscope. As stated, it is only with artificial elongation of the cell-walls that this change of direction is visible as a change in the double refraction, and it cannot be observed in natural elongation resulting from growth. Hence this natural elongation by growth is by no means to be regarded as a single plastic extension.

BONNER wondered why it is that in natural elongation there is no change in the double refraction. The answer is that during growth new transverse micelles are inserted in between the old ones. Hence it must be concluded that intussusception is an invariable accompaniment to the enhanced plasticity during growth. G. VAN ITERSON [288] established that even with the enormous lengthening of the cell-walls of the sporangium-stalk of *Pellia epiphylla*, which may reach 20 to 90 times its original length in a few days time, the tubiform pattern was maintained. In filaments of Gramineae, however, where growth is even more rapid, as these may attain 5 to 8 times their original length within an hour, FREY-WYSSLING observed a changed direction of the micellae. In this instance growth is apparently so rapid that intussusception cannot keep pace with it.

Although much has thus come to light concerning the structure and the growth of the cell-wall, our thirst for knowledge is not yet satisfied. The next question that arises concerns the nature of the auxin which results in a shifting of the cellulose micelles.

In this connection it must be borne in mind that for this action only minute quantities of auxin are required. BONNER and THIMANN calculated that the formation of 1.5×10^5 glucose residues, or approximately 70 cellulosemicelles corresponds to the action of one molecule auxin -a. Hence it can be no ordinary chemical action, as it is obvious also from the fact that, as already stated, both auxin and hetero-auxin promote growth. Moreover, in one of the artificial auxins indole-propionic acid, one isomer is 30 times stronger than the other. But there is even more convincing evidence of the complicated action of auxins, namely that as its concentration is stepped up a point is reached beyond which no increase, but a decrease of growth ensues. So there is an optimum in auxin concentration, and it will be seen later that in different parts of a plant, such as the root and the stem, this optimum may lie in a totally different level.

Hence we cannot but assume that auxins do act by way of the living material of the plant. In this connection the protoplasm will come to mind first, but in more recent years there have also been investigators who regarded the cell-wall as a living part of the cell. J. VON WIESNER [727] and much later B. HANSTEEN-CRANNER [232] argued this assumption. More recently BONNER and also SÖDING pointed to the phosphatides which are present in the boundary layer of the protoplast and spread themselves over part of the micelles of the cellulose skeleton. RUGE [559] argued a direct influence of auxins on the intermicellar substance of the wall.

But how are we to picture this action of auxin on the living matter, whether protoplasma or cell-wall phosphatides? It is understandable that physiologists of the school of PFEFFER such as FITTING [178] and BOYSEN JENSEN [69] still remain inclined to believe in the "Auslösungstheorie" and to view auxin as a stimulating substance "par excellence". The minute quantity required for the action would naturally lead to this assumption, though the fact that auxins are consumed during their action may seem to argue against it.

The school of F. A. F. C. WENT [709] opposes the application of the "Auslösungstheorie." They hold the rather more sceptical view that the idea of a stimulus does not really explain matters and is no more than a bogus explanation which may put the brake on further research. This view, adopted by F. W. WENT, was again opposed by L. JOST [305], who retorted that "WENT's Ausführungen über den Reizbegriff zeigen dass er diesen schwerlich an der Quelle studiert hat." (WENT's expositions on the concept of stimulation show that he can scarcely have studied this at the fountain).

In 1933 BONNER expressed the assumption that auxins were active only in aerobic respiration. This is in keeping with the results obtained later by H. E. VAN RAALTE [526].

By experiments with root tips VAN RAALTE found that an increased consumption of oxygen was accompanied by an increased consumption of auxin, whilst inhibition of respiration by means of hydrocyanic acid seemed to result in a decrease of auxin consumption. BONNER's assumption that auxin stimulated respiration was not proved by KÖGL's experiment with pure auxin, but these experiments were continued in America and led K. V. THIMANN and his collaborators to view auxin as part of a respiratory system. This system acts when succinic acid or malic acid is present and yields approximately 10% of the carbon dioxide liberated by the *Avena* coleoptile. The obvious conclusion is that auxin- a functions as a co-enzyme in this respiratory cycle which supplies the energy required for the elongation of the cells during growth. More recently BONNER and collaborators have concluded that auxin-a is a component of an enzyme, phosphatase.

It has been asked whether the old assumption of F. W. WENT, "without auxin no growth" has been strictly proved. According to S. STRUGGER [637], any factor which changes the ionisation degree of the protoplasma is a primary factor for growth. This author does not regard auxin as such, as it exerts only an indirect influence on the condition of the protoplasm by means of the metabolism of the cell. STRUGGER based this view on the doubly peaked curves, which he observed in graphs representing the influence of the concentration of H-ions on the growth of roots and hypocotyls. For these curves showed a certain resemblance to the two-peaked curves representing the influence of H-ions on the swelling of colloids. He considered that all growth began with a swelling of protoplasm. This view calls to mind the observations

of G. A. BOROWIKOW [61] of over 30 years ago. STRUGGER expressed the opinion that as a result of this swelling turgor would be increased and the cell-wall elastically extended. He thus held a view opposite to that arrived at by HEIJN and FREY-WYSSLING, mentioned above. Moreover, is it a proven fact that these doubly peaked curves do occur in growth? BONNER denied this and asserted that the influence of H-ions accounted to no more than an inhibition of the dissociation of auxin-a, which is a weak acid; the undissociated acid alone being active in growth. BONNER'S view was confirmed by the work of various investigators, though others opposed it. This gave rise to an investigation by ANNA M. VAN SANTEN [568] to test the conflicting opinions. Using the same object as did STRUGGER, the roots of *Helianthus*, she proved his explanation to be incorrect since double peaked curves do not occur. The p_H is of importance to growth only if a growth-promoting substance in the form of an auxin salt is present.

Formerly it was impossible to submit evidence for the hypothesis: "without auxin no growth" to a searching test, as there were no means of extracting small quantities from the plant or of determining them. For such determination recourse can now be had, in addition to the Avena-method already described, also to the "pea test." Herein slips of epicotyls of peas, which have been split lengthways, curve inwards even with very diluted auxin-solutions. Then there is the method of THIMANN [649] and SCHNEIDER [580], in which the *Avena*-coleoptile is split into four parts. Even with solutions of hetero-auxin of a concentration of 10^{-10} a decrease of the negative curvature arises, which gradually passes into a positive curvature at a higher concentration.

As regards extraction, this has become a complicated affair. During the first few years of auxin-research only the diffusion method, described above, was known. Later extraction was carried out with organic solvents, such as alcohol. ether, chloroform, provided that these were free from oxidizing mixtures. It was then found that the quantities obtained by these methods did not agree and, in addition to these quantitative differences, WENT also gave prominence to qualitative differences between the products which he termed "bound auxin" and „free moving auxin". The former seems to arise from the latter, but the conversion is not reversible. Bound auxin can be obtained from the plant only by means of extraction, whilst the free moving auxin is obtainable either by diffusion or extraction. The difference in other properties will be referred to again later.

In view of the foregoing it could be established that if by means of the most sensitive methods, growth could be still observed auxin was present in the cells.

As regards the transport of auxins, it was established straight away that this translocation occurred in strictly polar fashion. In connection with his polarity theory, of which will be dealt later, WENT assumed that there was a gradient of electric potential from the tip of the stem to the

tip of the root, the former being the negative, the latter the positive pole.

H. G. VAN DER WEIJ [716] studied this translocation of auxin in coleoptile-cylinders in a normal position as well as placed upside down. He concluded that in actively living parts the translocation is strictly polar and may go up against a concentration gradient, though in narcotized parts polarity disappears.

Although in experiments with abnormally high auxin-concentrations some investigators believe they observed an acropetal or crosswise transport, VAN DER WEIJ's conclusions were later fully confirmed by F. W. WENT. Polar transport was observed also in other objects, but its cause is still totally unknown.

During the past two decades the number of publications on auxins and their action has become so immense and the branches of plant physiology, where the auxin problem makes itself felt, are so numerous that a survey of the matter is by no means easily made. It would seem best to begin by following the development of the conflict between the so different views of BLAAUW and F. W. WENT. To do so, mention must be made first of the place where auxins are formed.

At first all that was desired was a closer definition of the place of formation in the coleoptiles themselves. H. G. DU BUY [102] and NUERNBERGK [467] succeeded in establishing that upon the removal of about 150 μ of the tip no further auxin diffused into the agar by the remainder of the coleoptile. After some time, however, a change sets in, for, as already stated by ROTHERT [554] in 1894, after decapitation a regeneration of the physiological tip occurs, or, as it is put nowadays, decapitated coleoptiles will resume the formation of auxin.

In 1937 F. SKOOG [608] expressed the novel view, that in the tip of the coleoptile no fresh auxin was really produced, but that there took place activation of the so-called "precursor", i.e. the inactive form of auxin already present in the seeds and transported to the tip of the coleoptile. Earlier N. CHOLODNY [113] had demonstrated that seeds contain a great deal of auxin or its precursor in its tissue. If POHL [513] eliminated this inactive auxin from the seeds by means of diffusion, the coleoptiles grown from these seeds showed symptoms of strong inhibition of growth, which was remedied by the artificial supply of auxin. Furthermore POHL draws attention to the contrast between the coleoptiles from Gramineae, where illumination seems to have no effect on the auxin production, and the seedlings of other species of plants, where it does have an effect and the formation of auxin seems to be associated with photosynthesis.

What about other objects? It is impossible to discuss all the various observations, but it may be said generally that all growing parts of higher plants contain larger ot smaller quantities of growth-promoting substances, and in particular of auxin. They have not been found in the Fungi and this may have some connection with the different composition of the cell-walls of the latter, which mostly contain chitin. Hetero-

auxin, however, has been found in Fungi, but whereas it is known that in higher plants this substance can serve as growth-promoting substance its function in the Fungi is still unknown. In the following chapter the function of bios substances in this respect will be dealt with.

The concentration of auxin in the various tissues is also a problem that is being studied a good deal. With the oat-coleoptiles this had already given rise to a great deal of controversy. Originally WENT believed that it was possible to explain the grand period of growth, the gradual increase to a maximum with subsequent decrease, as arising from two factors, the quantity of auxin and that of the material required for the extension of the cells, or "food factor". He thought that the former diffused from the tip of the coleoptile to the base, while the latter was transported from the endosperm into the coleoptile. The maximum of growth would then be found in the region where the optimum quantities of auxin and food-factor were present.

Later on, when it was possible to isolate auxin better and more accurately by extraction by means of chloroform or ether (see also what has been said before, regarding the two kinds of auxin), this conception proved to be too simple. Moreover, the fact that yet other factors cause the cell-wall to change in properties as it grows older, has to be taken into account.

Generally speaking, in seedlings others than those of cereals two types may be distinguished. In one an apical auxin-centre is present, in the other the auxin and probably its production is spread overall the growing parts and decapitation, therefore, affects growth but little.

It would, of course, be possible to mention all kinds of details concerning the auxins in buds, leaves, flowers and even parts of flowers. H. FITTING [178] showed as early as 1910 that water extracts of the pollinia of some tropical Orchids could cause the swelling of the gynostemium and he ascribed this to a hormone, a term used only in animal physiology until then. More than 20 years later F. LAIBACH [359] and MASCHMANN [410] again studied this growth-promoting substance from the pollen, which is probably identical with auxin, but discussion of this would lead us too far. The auxin from the roots, however, must be dealt with in greater detail, because it evinces various pecularities.

As the reader is no doubt aware, the zone of elongation in the roots lies at a few mm. distance from the root tip; now what about the auxin production in the root?

In connection with the polarity theory, referred to above, F. W. WENT had assumed a drop in electric potential from the tip of the stem to that of the root. in view of the acid character of some auxins, one might be tempted to assume that transport is explainable in this way, but since only the undissociated auxin-a is active there would not seem to be any point in this. Be this as it may, the auxin transport has a strictly polar character, as was argued above. Are we to conclude from this that the auxin in the root is derived from the stem, or is there in the root also

a polar transport from root-tip to root-base? N. CHOLODNY [113], who is bracketed with F. W. WENT as the founder of the auxin theory of tropism, as early as 1924, attempted to explain the positive geotropism of the root by means of the auxin.

CHOLODNY demonstrated experimentally that the auxin transport occurs from the tip to the base of the root and not vice versa, whilst THIMANN [649] in his extraction experiments by means of chloroform observed an increase of auxin towards the root tip. This was, therefore an argument for the existence of a production centre in the root tip. Indeed, BOYSEN JENSEN [69] succeeded in getting auxin from isolated root tips, placed on glucose-agar, to pass into the agar. In contradistinction to THIMANN, he even succeeded in causing more auxin to be diffused from them in 20 hours than it was possible to obtain by direct extraction with chloroform. This increase of auxin in isolated root-tips placed on glucose-agar was confirmed by the work of M. H. VAN RAALTE [526].

Although some investigators, such as H. FIEDLER [172] still raised objections, the production, or at least the activation of auxin in the root-tip seemed practically certain from the foregoing. But the problem was not yet solved, for CHOLODNY observed that after decapitation of the root-tip the stump grew more vigorously and that this increased growth could be inhibited by placing the tip of an oat-coleoptile on the stump, while its own tip had a similar, though slightly weaker, action. Hence there was the curious case of a growth-promoting substance inhibiting growth. This was confirmed by experiments by BOYSEN JENSEN, who compared the action of a solution of hetero-auxin on intact roots of *Vicia Faba* with the action on decapitated roots. In both instances the growth rate was found to decrease, but to cause a 50% decrease a much stronger solution of hetero-auxin was needed with the decapitated than with the intact roots.

The question then arose as to how it was possible for the auxin to have such an antagonistic action on stems and roots. A. Th. CZAJA [129] formulated a very involved theory on this point, which, although most ingenious, is not discussed here, because no more than a year after its publication it was completely refuted by other investigations.

In 1936 BOYSEN JENSEN suggested the possibility that the root might be so sensitive to auxin that this substance could promote its growth, but did so only in much lower concentrations. Proof of this assumption was soon forthcoming. In the same year M. GEIGER-HUBER [196] and E. BURLET [97] found that with concentrations of hetero-auxin between 2.86×10^{-5} snd 2.86×10^{-10} mol. the inhibiting action on the growth of maize roots ceases and gives way to a promoting action. With 2.86×10^{-11} mol. (0.005 γ per Litre) such promotion reaches its maximum. In the same year again FIEDLER [172] and H. A. AMLONG [9] found a similar promotion of growth with corresponding concentrations, the former in maize, the latter in *Vicia Faba*, the broad bean.

From this GEIGER-HUBER and BURLET concluded that auxins are

equally essential for the growth of roots as for that of the stem. The experiment made by FIEDLER, who found that isolated maize-roots grew although it was impossible to find a trace of auxin in them, may probably be explained by the fact that it is exceedingly difficult to demonstrate the presence of the minute quantities of auxin necessary for the growth of roots. Later this assumption was justified by the fact that VAN OVERBEEK [487] and BONNER [59] proved the presence of auxin in pea-roots grown in vitro.

In passing it should be noted that this discovery by GEIGER-HUBER and BURLET is of great importance in connection with the explanation of the positive geotropism of the roots. This will be referred to again later in the discussion on the action of gravity. But it useful here to mention the instructive comparison used by GEIGER-HUBER and BURLET to explain how minute the concentration of auxin has to be if it is to have no action on the growth of roots. Supposing it was desired to dissolve one gram of hetero-auxin in so much water that its growth-promoting action was obviated, 200×10^6 M^3 would be required, a quantity which could be conveyed by 400.000 railway trains each of 50 cars, each holding 10 tons of water.

To revert to phototropism, in his publication of 1928 F. W. WENT concluded that no compromise was possible between BLAAUW [51] 's theory and the auxin theory. In his opinion the attempt by VAN DILLEWIJN [138] to reconcile the two must be regarded as having failed. All the same, repeated attempts were made to effect such a compromise and some of them must be discussed.

In 1933 J. VAN OVERBEEK [487] published his investigations on phototropism and lightgrowth response in seedlings of *Raphanus sativus*. He used seedlings which had been adapted to darkness by a stay of 10 hours in a dark room. Growth in such seedlings is not confined to the tip, but is spread all over the hypocotyl. The auxin required for growth is formed in the cotyledons.

The lightgrowth response cannot be fully explained by inhibition of the auxin production. The fact that the response to the action of the auxin changes as a result of illumination must also be taken into account. VAN OVERBEEK considers that the positive phototropic response is due not only to the unequal distribution of the auxin-a, but also to the fact that the reaction to this auxin in the coleoptile is then distributed in such a way that the shaded side is much more sensitive to it than the light side. In this instance, therefore BLAAUW's lightgrowth response and WENT's auxin theory do not conflict, they are fundamentally complementary in explaining the phototropism of the hypocotyls of *Raphanus sativus*.

VAN OVERBEEK is of opinion that approximately half of the phototropic curvature of these hypocotyls is due to the uneven distribution of the auxin-a, the so-called "CHOLODNY-WENT effect", the other half of the lightgrowth response being brought about by the decreased sensitivity to light, the so-called "BLAAUW effect".

In *Helianthus globosus* matters are different; as in these hypocotyls the difference between the respective lightgrowth responses to the light-intensity at front and back can explain the resulting curvature, it may be said that BLAAUW's theory provides sufficient explanation in this case.

The position is different again with *Avena sativa*. In 1928 A. BEYER [47] observed lightgrowth response in this plant without any phototropic curvature, though the reverse was found by CHOLODNY a few years later. According to WENT, the lightgrowth response is caused in this instance by a decrease of about 20% of the auxin diffusing from the tip of the coleoptile. This must be the so-called long reaction, which arises approximately an hour after the optimum illumination. As we saw above, there is also the short, or basal, reaction, which sets in within less than half an hour after illumination of the base of the coleoptile, provided that such illumination occurs with plenty of light.

When VAN OVERBEEK made tests with decapitated oat coleoptiles, by placing one-sidedly a small cube of agar containing auxin-a or hetero-auxin on the stump, he found there was a difference between light and dark with the former, though not with the latter agar-cube. With the coleoptiles with auxin-agar curvature was much weaker after all-sided illumination than in the dark, which fact VAN OVERBEEK ascribed to the destruction of auxin-a in illuminated plants. In that same year 1936 KÖGL [339] and his collaborators C. KONINGSBERGER [342] and HANNI ERXLEBEN [161] found that in a weakly acid solution auxin-a is in equilibrium with its lactone, which substance, after illumination with ultra-violet light, turns into luminauxin-a lactone which cannot promote growth. In visible light this inactivation would require a sensitizer, and in connection with the theoretical observations of E. BÜNNING [89], carotenoids would come to mind in this respect. There is a striking similarity between the spectral sensitivity of phototropism of the oat-coleoptile and the absorption-curve of carotene in ether. According to BÜNNING phototropism in the sporangophore of *Phycomyces nitens* also has some connection with the presence of carotene which may serve as a sensitizer.

The experiments by KÖGL and his collaborators gave rise to investigations by V. J. KONINGSBERGER [343] and B. VERKAAIK [672], who used de-seeded and decapitated Avena-coleoptiles. These contain no auxin-a and yield the basal-reaction after prolonged, one sided illumination if auxin-a is placed on top of the stump, though not if, instead of auxin, hetero-auxin is used. Hence the authors concluded that there is reason to ascribe the phototropic basal response to a partial inactivation of auxin-a under the influence of light.

W. F. F. OPPENOORTH JR. [478] later studied the apex reaction of such coleoptiles with special reference to the effect of much smaller quantities of light-energy which give rise in the main to the so-called first positive curvature. He argued that auxin may play a part in phototropism in three ways:

(1) by photo-inactivation of the auxin-a lactone;
(2) by a change in the auxin-synthesis as a result of illumination;
(3) by lateral auxin transport.

Of the third possibility there is only circumstantial evidence, whereas of the first two direct proof is available. According to OPPENOORTH, though photo-inactivation may explain the inhibition of growth with the apex-reaction, it cannot explain the phototropic curvature, as the lightgrowth responses on both light and shadow sides are equal. Hence BLAAUW's theory cannot apply in the region of the first positive curvature; in OPPENOORTH's opinion lateral translocation of auxin-a must be playing a part.

The question as to the part played by the bound and the free moving auxin in relation to the processes of growth and phototropism cannot be dealt with here.

Much space has already been devoted to show the complexity of growth and phototropism even in the few objects hitherto subjected to investigation. This has shown the lengthy and laborious research which is required for every step forward in the analysis of these phenomena, as well as the great number of persons which is needed for this work.

In recent years the investigations on growth promoting substances in America are so numerous that it is impossible to mention them here. The emphasis on growth research has now shifted from Europe to America. In connection with the investigations on these growth promoting substances it may suffice to mention the names: ZIMMERMAN [755] and HITCHCOCK [260], AVERY [22] and co-workers, the Chicago group of KRAUS [346] and HAMNER [228], the U.S.D.A. group of MITCHELL [430].

A few words on the question, interesting from a physiological point of view, as to how it is possible for such divergent substances as heteroauxin and artificially prepared auxins, as napthyl acetic acid, and indolebutyric acid, studied by ZIMMERMAN and HITCHCOCK-, to have such a strong growth-promoting action, although chemically they are totally different from auxin-a.

E. VON GUTTENBERG [217] studied this question by extracting auxin from internodia of *Coleus* on which he had put indole-acetic paste. In determining the quantity of the latter substance in respect of auxin-a he took into consideration the fact that auxin-a resists treatment with acids, but not with alkalies, whereas the reverse is true of indole acetic acid. He found that internodia, on which indole acetic acid paste was placed, contain far more auxin-a than those without. VON GUTTENBERG concluded that indole acetic acid stimulates the production of auxin-a.

JE. OORTWIJN BOTJES [475] came to quite different conclusions in respect of the action of ethylene on auxinproduction, a subject which had already been studied by VAN DER LAAN [358]. Experiments of OORTas if WIJN BOTJES with epicotyls of peas and with tomato-stems showed that after having been in an ethylenous atmosphere these objects behaved

they contained more auxin. Cut coleoptile-cylinders of *Avena*, however, which had been in an atmosphere with ethylene, contained 50% less auxin than the controls. The conclusion of the author was that ethylene stimulates the consumption of auxin.

The evidence for enhanced auxin production or consumption by resp. indole-acetic acid and ethylene is however dubious and experimental research will have to determine whether these conceptions are correct or whether the view expressed formerly by F. W. WENT is to be preferred. The view of the latter is, that all growth substances natural as well as artificial ones have a direct action.

WENT and THIMANN in "Phytohormones" say: The primary growth promoting activity is connected with the presence of: 1, the double bond, or aromatic unsaturation; 2, a carboxylgroup, free, or if esterified, readily hydrolysable; 3, a ring system, either 5 membered (auxin-a and b), aromatic (naphtyl or phenyl), or a combination of both (indole, indene); 4 a minimum distance of at least one C atom between the carboxyl group and the ring; 5 a very definite steric structure, since in the one case studied the cis-compound is active, the trans-compound not". A hypothesis relating structure to activity has been given by WENT, KOEPFLI and THIMANN.

We may wind up by saying that mention should be made, of a few estimates of the number of molecules of auxin active per growing cell. For the *Avena*-coleoptile Bünning estimated this number at about 10^4 per cell, whilst E. C. WASSINK [701] believed it to be 3.6×10^4, GEIGER-HUBER and BURLET examined the sensitivity of isolated maize-roots to hetero-auxin and estimated that, with optimum growth, 4×10^5 molecules were active per cell. Hence these are all values of nearly the same order. Though this number may seem very large, it is but infinitesimal compared with the number of protein molecules of protoplasm. If large protein-molecules with a molecular weight of 68000 are taken into account, it will be found that 1 molecule of auxin is active on a number of protein-molecules which lies roughly between 10,000 and 1,000,000. This brings to mind PFEFFER's old view regarding stimulus action, which WASSINK in an attempt to translate it into the language of modern physica, denotes with a term derived from wireless technique, viz. amplifier-action. However, we must not forget the idea of an auxin function as a co-enzyme mentioned above.

Chapter IX

GEOTROPISM

(A) Former Views on Geotropism.

As stated at the beginning of the previous chapter, Ch. Darwin [132] found in 1881 that the perception of gravity was seated in the root tip, This fact made it obvious that there must be conduction of this stimulus to the zone of curvature a few mm. away. One year before publication of the 3rd edition of the Textbook by Hugo de Vries, W. Rothert [554] concluded from the fact that phototropic and geotropic curvature take a similar course that the tip of coleoptiles must also be most sensitive to gravity. Yet it was obvious that the zone below the tip is not altogether deprived of sensitivity to geotropic stimuli as was shown by the experiments of Darwin. The latter obtained geotropic curvatures even with decapitated coleoptiles.

Nevertheless, objections were raised by many workers to experiments with wounded plants, and this gave rise to the application of a new method by F. Czapek [130], who caused roots to grow in capillaries bent at right angles. Under those circumstances he likewise obtained the result that perception of the stimulus occurred in the tip, the reaction taking place approximately 2 mm. below the tip. A few years later F. Darwin [133] did the same with tips of coleoptiles and also found that the reaction in the zone of elongation was determined by stimulation of the extreme tip.

In 1904 A, Piccard [507] applied a totally different method. He used a centrifuge, in which the parts of plants to be tested were placed at an angle to the axis, in such a position that the tip and the zone below the tip were stimulated in opposite directions. Piccard himself used roots for this experiment, whilst H. von Guttenberg [217] later applied the same method to coleoptiles, where again the tips (3-5 mm.) of *Avena*, *Hordeum* and *Phalaris* were found to be much more sensitive.

At that period attention was devoted mainly to the perception of gravity. Czapek formulated a theory which calls to mind our modern views on auxins. According to him, processes of oxidation in geotropic stimulation led to the formation of homogentisic acid from tyrosine.

Homogentisic acid is closely related to phenylacetic acid, which is now known as an artificial auxin.

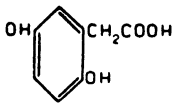


FIG. XXV. Homogentisic acid

A greater impression, however, was made at that time by the socalled statolith-theory, arising out of a statement made by F. NOLL [465] in 1902. This author suggested the possibility of submicroscopical structures in the boundary layer of the protoplast acting as statocysts. Organs for geoperception are present in various species of animals and consist of a small body with relatively large specific weight which exerts pressure on different sensory hairs according to the position of the animal.

Both G. HABERLANDT [222] and E. NEMEC [454] ascribed these phenomena of geoperception to visible constituents of the cell. For example, according to them the starch grains, particularly those in the starch-sheath, the rootcap and the columella of mosses function as statoliths. This theory gave rise to a number of publications, both for and against. A point raised was that there were plants and parts of plants which do not contain starch grains and are yet susceptible to geotropic stimulation, for instance the sporangophore of *Phycomyces nitens*, root-hairs, etc. In such a case both NEMEC and HABERLANDT ascribed the function of geoperception to the nucleus but then the question arises as to why this should not also play a part in the root. In fact, much more rapid and better response to gravity is seen with the starch grains than with the nucleus of these cells.

H. FITTING [178] and L. JOST [305] also opposed the theory in connection whith the results obtained in experiments with intermittent stimulation, which can hardly be made to fit in with HABERLANDT's views, as the perception of gravity was found to occur without removal of the starch grains. To overcome this difficulty HABERLANDT had to assume that removal of these grains was unnecessary, the exertion of pressure being sufficient.

A different approach to the question was made by experiments to ascertain whether roots still responded to gravity when deprived of their starch by some harmless method. To this end the root was encased in plaster or placed in a cold environment, whereupon it was found that the response to the stimulus of gravity disappeared simultaneously with the disappearance of the starch. Opponents to the statolith-theory objected, however, that under those circumstances the plant was no longer normal and did not react for that reason.

It is also possible to dissolve the starch by chemical means, for instance with the aid of potassium-alum. But when Mrs. RUTTEN-PEKELHARING [562] applied this method, the roots were likewise found to be abnormal and to yield traumatotropic curvatures, so that little is proved by insensibility to gravitational stimuli.

However, this last-named investigation is of greater importance on account of a result, mentioned above, that with stimuli of gravity

acting on the same kind of plant and under the same circumstances the product of the time of exposure and the active force is constant. Centrifuging experiments were made with oat-coleoptiles to ascertain how long they should be exposed to the action of a given centrifugal force to induce curvature in 50% of them. With *Avena sativa* the product of time and intensity was found to be constant within the range of centrifugal force of 0.08 and 58.43 g,. and with the root of *Lepidium sativum* this product-rule was likewise found to hold good within certain limits.

FITTING who worked with an intermittent clinostat, proved that the response of gravity is proportional to the sine of the angle of deflection from the vertical. Mrs. RUTTEN-PEKELHARING utilized this result in her experiments concerning the product-rule. By varying the intensity of gravity through using different angles of deflection she found that a barely visible curvature, the threshold value, also required a constant product of gravity and time of exposure.

This vividly recalls the product-rule for phototropism, found by BLAAUW [51], though the difficulty remains that gravity can act as an energy source only if acting on moving particles. This would be possible if the statolith-theory held good, but there are many drawbacks to this theory, as we have already stated. The colloidal protoplasmic particles, however, are also movable.

About 1911, as BOYSEN JENSEN [69] was carrying out his experiments concerning the conduction of the phototropic stimulus, he made some observations in regard to the conduction of geotropic stimuli and concluded that in this case also the stimulus was conducted by means of translocation of an still unknown special substance.

(B) Significance of Auxins for Geotropism.

The analogy to phototropism, mentioned above, led N. CHOLODNY [113] as well as F. W. WENT [710] to apply the auxin theory to geotropism. They considered that geotropic curvature might arise from an uneven distribution of auxin-a in the parts concerned as a response to gravity

Experiments of H. E. DOLK [140] brought the expected proof. He found that the direction of gravity was immaterial for the production of auxin in coleoptiles, but affected the distribution therein. Hence there is no geo-growth response comparable to the lightgrowth response, but the auxin accumulates along the lower side of the coleoptile. After half an hour the ratio between the respective quantities in the upper and lower side is approximately 1 : 2. This explains the fact mentioned above that not only the utmost tip, but also the parts situated more basal are sensitive to gravity, albeit in a lesser degree.

The auxin theory of CHOLODNY-WENT, therefore, provides an excellent explanation of the geotropic response of oat-coleoptiles, though the way in which the uneven distribution of auxin arises re-

mains unexplained. In regard to this question a theory was formulated earlier by CHOLODNY and also, independently, by J. A. SMALL [611], in both of which electricity played a part.

In 1925 L. BRAUNER [71] was the first to mention in this respect the "geo-electric effect". By this is meant the phenomenon that in parts of plants in a horizontal position, whether they are alive or dead, the lower side becomes positively charged in respect of the upper side. To explain this fact, which had already been noted by J. C. BOSE [63], reference is usually made to cataphoresis. It is assumed that a shifting of electrolytes through the cell-membranes occurs, in which the anions are strongly adsorbed, causing the cell-wall to become negatively charged, whereas the cations are removed to the lower side of the organ as a result of the action of gravity. It is questionable whether this phenomenon, which may also be obtained in model experiments with parchment, has in fact any connection with the perception of gravity.

BRAUNER placed plants in a strong electric field which must cause a similar shift of ions. The result was that between two aluminum-electrodes in a field of direct current of 640 volt per cm. the positively geotropic root curves towards the negative pole and the negatively geotropic oatcoleoptiles curve towards the positive pole. Hence an explanation in this direction is feasible, though it cannot be regarded as certain, one reason being that the essence of the entire auxin translocation still remains totally obscure.

The question remains as to what causes the antagonistic response of roots and stem to the stimulus of gravity. As pointed out in the previous chapter, this is regarded nowadays as being a difference in sensitivity to the action of auxin between the root and the stem. The concentration of auxin, which causes the optimum rate of elongation in the root, is so minute that the increase of auxin at the lower side, resulting from the horizontal position, suffices to cause an accumulation beyond this optimum concentration. In consequence, the lower side of the root will grow less than the upper side, with the result that positive geotropic curvature arises. That this must be the correct explanation is strongly supported by the observation by H. U. AMLONG [9] that decapitated roots on which agar-blocks containing a low concentration of auxin are placid, show negative geotropic curvature.

So far there has been discussion only of the action of gravity on the so-called orthotropic organs, like the main root and the stem, which respectively turn vertically up and downwards under its influence. But the external appearance of plants is determined chiefly by the position of the so-called plagiotropic organs, lateral axes, leaves and flowers, which in turn is determined by internal factors as well as by external ones.

In the Textbook by HUGO DE VRIES we find the view expressed that besides by geotropism and phototropism the position of the organs of the plant are determined by epinasty and hyponasty. Epinasty causes

the upper side of organs to become convex, whilst hyponasty causes convexity of the lower side. This more or less implies dorsiventrality of the organ, which in general may be observed also morphologically. But lateral roots, which place themselves at a certain angle to the main root, have a radial structure. So have rhizomes growing horizontally.

During the past 50 years there has been no lack of attempts to gain further insight into his plagiotropism which L. JOST [305] in 1923 still called one of the most obscure chapters of physiology. The terms epinasty and hyponasty used by DE VRIES, were in reality no more than descriptions of facts.

In further research an important part was played by the clinostat. HUGO DE VRIES had used a lateral position in his experiments, in which a leaf was placed vertically in such a way that the stalk and the main rib were horizontal and gravity acted in a different direction from what DE VRIES termed purely autonomous epinasty or hyponasty. The use of the intermittent clinostat by H. KNIEP [333] constituted an improvement in method. Here the plant or its organ were placed so that two lateral positions followed each other intermittently, hence the geotropic stimulus was eliminated, or rather cancelled by the next stimulus and only any nastic stimulus that might be acting autonomously could take effect. For this kind of research it is important to use a clinostat which runs with perfect regularity. Such an apparatus with electrical drive was constructed by PH. VAN HARREVELD [238].

In such investigations with plagiotropic organs a sharp distinction must be made between a truly dorsiventral organ like a leaf and a plagiotropic, radially symmetrical organ such as the lateral branches of *Asparagus plumosus*. With the former it is of importance which side is up, whereas this is of no consequence with the latter that are able to right themselves by one single curvature from any position to that of equilibrium, which dorsiventral organs can achieve only after torsion. Later the development of this dorsiventrality will be discussed.

In the experimental plant used by KNIEP, *Lophospermum scandens*, one of the Scrophulariaceae, whose leaf stalks function like tendrils, curvatures arose even after elimination of geotropic stimuli. From this KNIEP concluded that there were autonomous curvatures independent of gravity, which he termed epinastic curvatures. Similar to the behaviour of this object, which is dorsiventral also from an anatomical point of view, is that of some organs, which anatomically speaking, have a radial structure. Other organs of the last-named type behave as if they were physiologically radial after a shorter or longer stay on the intermittent clinostat. In such instances F. RAWITSCHER [531] spoke of labile dorsiventral organs in which dorsiventrality is induced or, in other words, caused by gravity. RAWITSCHER denotes this phenomenon by the term geoepinasty. Though gravity provides the stimulus for the arising of the property, the ensuing direction of curvature is independent of the direction of this force; to use PFEFFER's terminology, it is a nastic response induced in youth and not purely autonomous.

Besides gravity, there are also other external factors which may bring about this induction. For example, the plagiotropism of the stems of *Tradescantia fluminensis* is found to be epinastic only in light. In the dark the shoots are orthogeotropic, which fact is due to a tonic influence of the illumination. Experiments carried out by INA E. UIJLDERT [668] in the laboratory of F. A. F. C. WENT showed that the epinastic curvature must be ascribed to an asymmetric auxin translocation caused by gravity.

Contrary to these views concerning epinasty and hyponasty, which in a sense are a continuation of the work of HUGO DE VRIES, is the conception of H. LUNDEGÅRDH [393], which was elaborated by W. ZIMMERMANN [756]. LUNDEGÅRDH's starting-point was the study of the stems of *Coleus* which are what RAWITSCHER would call labile dorsiventral. After a couple of week's stay on the clinostat, and sometimes after an even shorter period, *Coleus* no longer shows any dorsiventrality and the epinastic curvature fails to appear. LUNDEGÅRDH was inclined to regard the epinasty of such objects as an after-effect of gravity and to explain the position of equilibrium by the influence of gravity on the longitudinal growth. For this he had to assume that these organs are both positively and negatively geotropic and, moreover, that gravity has a tonic effect on longitudinal growth. In regard to this second assumption, it should be borne in mind that according to DOLK [140] there is no geogrowth response and that action of gravity does not alter the auxin production. In my opinion this is an argument against Lundegårdh's views.

W. ZIMMERMANN views were in keeping with those of LUNDEGÅRDH. Runners of *Ranunculus repens* constituted his chief experimental material. If these were placed on the clinostat, plagiotropism disappeared after a few days and they would continue to grow horizontally. When the clinostat was stopped, first a negatively geotropic curvature would set in and the runners would form an angle of approximately 60° with the horizontal. Subsequently there would be yet further curvature, so that after approximately 3 hours a position was attained in which the runner formed an angle of 25° with the horizontal. According to ZIMMERMANN, the first curvature proved the presence of negative geotropism with a short reaction time, whilst by the second curvature the existence was proved of positive geotropism with a longer reaction time. The ultimate position would be reached when the joint action of these two was balanced by the so-called tonic action of gravity, which affected the runner longitudinally. This may also be expressed by saying: the sine-rule does not hold good either for positive or for negative geotropism, or for both, in consequence of the tonic action of the length-component.

P. METZNER [422] tried to provide these views, which in the present writer's opinion are not very clear, with a mathematical basis. He wished to find a better means of formulating the antagonistic tonic influence, but it is highly questionable whether the attempt can be regarded as successful.

The complexity of these phenomena may best be shown by an somewhat more detailed discussion of a single case, the curvature of the pedicel of the poppy, *Papaver Rhoeas.*

HUGO DE VRIES had observed that upon removal of the bud the pedicel righted itself and from this fact J. VON WIESNER [727] concluded that the weight of the bud caused the curvature. VÖCHTING [684] demonstrated this to be incorrect, since introduction of a counterweight fails to prevent the righting. According to him, removal of the bud or even of the ovary from the bud causes the positive geotropism, to change into a negative geotropism hence he regarded the ovary as the susceptible organ. He considered that the stem was negatively geotropic at the base and positively geotropic at the tip, with a neutral zone in between. In his opinion this neutral zone moved more and more towards the tip until just before the flower opened the positive geotropism had altogether disappeared.

In 1921 HELENE SCHULZ [590], one of FITTING'S pupils, made special experiments to ascertain whether this conception of the ovary functioning as perceiving organ was correct. This was not found to be the case. According to this investigator, the influence of the bud has a tonic character and its removal disturbs normal development. In other words, according to HELENE SCHULZ there is indeed action by gravity, but besides this there is also a nastic factor such as the clinostatic experiments appeared to indicate.

Later FITTING himself ascertained whether besides decapitation there may also be other factors which change the geotropic tonus. From the fact that in the dark buds right themselves more quickly he concluded that with both illumination and non-illumination the stem was the most important part. Instead of the ovary being the organ for geoperception, it now seemed as if the pedicel was the organ for photoperception.

Finally, RAWITSCHER denied any positive geotropism in the bud of the poppy. He considered that the active curving of the tip might also be due to dorsiventral epinasty, which we shall find later in a number of seedlings. Careful experiments with the intermittent clinostat led RAWITSCHER to this conclusion, which does seem an attractive one, although it was opposed by ZIMMERMANN.

(C) Twining Plants.

It is at this juncture that twining plants must be discussed, for no matter how divergent the opinion on the phenomenon of winding, gravity is always found to be a more or less important factor.

In the Textbook by HUGO DE VRIES the view was expressed that winding was due in the first place to circumnutation by the tip of the stem. This circumnutation was supposed to be caused by the fact that the rate of longitudinal growth was not the same at all sides of the stem.

Sometimes one side will grow faster, sometimes the other or to express it more accurately the faster growing side gradually circles the stem, so that the tip moves upwards in a spiral. For the side which grows faster, of course becomes the convex one, whilst the side which grows more slowly becomes concave. This circumnutation, which is autonomous or independent of external factors, is according to DE VRIES accompanied by a negative geotropism in the parts of the stem further away from the tip, so that only vertical supports may become entwined. In short the point of view expressed by DE VRIES in 1873.

In his Textbook HUGO DE VRIES therefore ignored the work of F. NOLL [465], who in 1885 had formulated the theory of lateral geotropism in keeping with the view of F. J. BARANETZKY [29]. According to this theory, one side of the stem close to the tip was stimulated to stronger growth by the force of gravity. It was assumed that with plants twining leftwards the right side, and with those twining rightwards, the left side grew more strongly and in this way became the convex side. The side which grew more strongly was assumed to run spirally round the stem and to move constantly towards the tip. Hence in contradistinction to autonomous nutation, NOLL assumed a non-autonomous lateral geotropism. This conception fitted in with the opinion prevalent at the time, that on the clinostat twining came to a standstill and also with the fact mentioned above, that twining is possible only round a practically vertical support.

Yet some objections were raised. W. NIENBURG [460] argued that the cessation of twining on the clinostat proved nothing concerning the existence or non-existence of an autonomous movement, whilst also C. F. B. BREMEKAMP [73] laid more emphasis on the autonomous nature of the phenomenon.

The clinostatic experiments constituted the pivot on which everything hinged. V. ULEHLA [663] caused the tops of twining plants to rotate round the horizontal axis of the clinostat and found that they became straight, after which they were exposed again to one-sided gravitational action. From his results, which were confirmed later by L. JOST [305] and GERDA VON UBISCH [662], it may be concluded that there is both a negative and a lateral geotropism, the former seated in the base, the latter in the region of the tip, each being characterized by its own special presentation-time which in one and the same plant for instance amounts to about 90 seconds for the negative, and to approximately 10 seconds for the lateral geotropism.

H. GRADMANN [208], however, explains twining by a so-called "Ueberkrümmungsbewegung" (overdoing curvature). He considered that the stem was only negatively geotropic, though the accompanying response was so intense that the tip curved beyond the vertical and was thus once more subjected to the gravitational stimulus, as a result of which it passed the vertical again. But GRADMANN had to admit that the existence of species of plants which regularly twine either to the left or to the right

was proof that besides this negative geotropism there must be yet another directive force. In the present writer's opinion GRADMANN's considerations point to a lateral geotropism in disguise.

RAWITSCHER [531] took the field against all these authors who, with the exception of BREMEKAMP, are of opinion that twining cannot be a purely autonomous movement, and he tried to get the old view of CH. DARWIN [132] accepted again. In his research RAWITSCHER used lapse-time pictures of different objects. With branches of *Pharbitis hispida*, one of the Convolvulaceae, it was found that this object does begin to twine when the support around which it had wound was removed, though there is no trace of the "Ueberkrümmungsbewegungen" assumed by GRADMANN.

RAWITSCHER went further and did careful experiments to ascertain whether it is a fact that on the clinostat twining comes to a standstill. He concluded that this was not so, which view was confirmed in the laboratory of F. A. F. C. WENT in Utrecht. RAWITSCHER even gave a description of cases where twining was maintained for three weeks on the clinostat and produced further evidence of autonomy. The lateral shoots of *Pharbitis* twine round a support which forms an angle of about 60° with the vertical a case where lateral geotropism can hardly play a part.

Asparagus plumosus, a plant specially studied by RAWITSCHER, undoubtedly possesses an autonomous movement in his opinion. This was given much prominence by him in his first publication, though in his later summary, "Geotropismus der Pflanzen" (Geotropism of plants) he retracted and admitted the existence of lateral geotropism in species such as *Pharbitis hispida*. In a sense it may be said that this author arrived at a compromise in that he considered there were species of plants for instance *Asparagus plumosus* without lateral geotropism as well as plants like the Convolvulaceae, where this kind of geotropism does play a part.

After RAWITSCHER's book there appeared the thesis by JET. C. KONING [341] written in the laboratory of F. A. F. C. WENT. This author views the movement of the freely growing tip of twining plants, which movement she terms rotation, as separate from the twining round a support. She makes a distinction between three kinds of rotation.

(1) an autonomous rotation with small amplitude, for which she uses the term cyclonasty, a term before used by BREMEKAMP, though in a somewhat different sense;

(2) a rotation with wide amplitude, which is enhanced by the tonic influence of the one-sided action of gravity;

(3) an asymmetric rotation, which occurs in plants with lateral geotropism and is under the influence of the one sided action of gravity.

Winding by virtue of autonomous rotation with small amplitude, though theoretically possible, does not occur in nature. But winding by virtue of rotation with wide amplitude, which is found to be impossible when the one-sided action of gravity is excluded ,is the rule in plants without lateral geotropism.

Finally, there is winding by virtue of lateral geotropism, caused by

the influence of gravity, which plays the chief part in the twining of species belonging to the Convolvulaceae.

The last-named investigator also carried out some experiments with auxin and concluded that in *Pharbitis hispida* growth of the stem is not influenced by the auxin formed in the leaves. However, the fundamental question concerning the part played by auxin in the typical, gradual shifting of the zone of strongest growth in twining plants is still totally obscure.

CHAPTER X

SIGNIFICANCE OF INTERNAL AND EXTERNAL FACTORS IN GROWTH AND DIFFERENTIATION

(A) Polarity and Dorsiventrality.

In the development of plants with a higher organisation differentiation occurs, in which a part is played by the contrast between apex and base or between upper and lower side. The former is denoted by the term polarity, the latter is more especially termed dorsiventrality.

Polarity is a property not only of the individual plant as a whole, but also of its parts and even of the cells which compose them. The last-named point is clearly shown in transplantation which generally succeeds only with a normal position of the parts implanted, as otherwise abnormalities arise, which H. VÖCHTING [684] demonstrated experimentally as early as 1892.

The subject is not dealt with in the Textbook by DE VRIES, though SACHS had mentioned it in his book "Vorlesungen über Pflanzenphysiologie", published in 1882. The question of primary interest from a physiological point of view is whether this polarity is present from the beginning or whether it is caused, or in physiological terms induced, by some external factor. In the opinion of VÖCHTING polarity was fixed and inherited, whilst PFEFFER assumed it to be induced by intercellular correlation. SACHS on the contrary took it for granted that there was an induction by external stimuli which was gradually fixed. In the first years of this century KLEBS [327] voiced the idea that polarity remained changeable.

The development of the embryo of the Phanerogams from the fertilized egg-cell is not a suitable object of study in this regard, because the surrounding cell-layers may naturally have an inductive action. That this is actually the case is shown by the place of the root which is always turned toward the micropyle of the ovule.

In the egg-cells of Fucaceae, which after fertilization begin their further development floating in seawater, this problem was studied by H. WINKLER [734] with *Cystosira*. WINKLER found that in the first cell-division light must be the inducing factor and that the previous position

of the egg-cell in the oogonium was of no importance, for the first cell-wall arose at right angle to the direction of the incoming rays of light.

A later investigation by H. KNIEP [333] showed that about 10 hours after the fertilization of *Fucus* a couple of hours light-action was required before polarity was definitely established. It had been stated that the direction of the light rays had a determining effect on the position of the spindle in the first division of the nucleus and thence the formation of the new cell-wall. KNIEP, however, noted that the bulging of the rhizoid cell, formed in this first division, arose long before there was any hint of nuclear division. Hence polarity in the fertilized egg-cell must have arisen already before the first nuclear division.

Therefore, the genotype, i.e. the complex of hereditary properties of these Fucaceae, determines the arising of polarity, though external circumstances such as illumination determine its direction. As H. KNIEP had observed that germination occurred also in the dark, he was of the opinion that under those circumstances the direction of the axis of polarity was either accidental or was determined by the nutrient medium.

In this connection the investigations of E. KNAPP [332] are of interest. They were of a similar nature as those of KNIEP: when experimenting with *Cystosira* KNAPP found that polarity became fixed by illumination over a period of two hours, which could be inferred from the bulging of the rhizoid cell. Centrifuging likewise had a determining action and subsequent one-sided illumination fails to have any effect. However, KNAPP found that the direction of polarity was determined also in the dark, though it might be changed by illumination immediately afterwards. In the dark, the point where the fertilizing spermatozoid has entered the egg-cell, which place may be recognized by the uneven surface, has a determining action on the formation of the bulge of the rhizoid cell and thus on the direction of polarity. Whether the place where the spermatozoid enters was indeed entirely fortuitous is, of course, a moot point.

According to ANNIE M. HURD [284] the effect of illumination is caused by wave lengths of 4000-5600 Å, mainly also by the blue rays.

W. NIENBURG [460] studied the question whether in illumination it is the direction of the light rays or the decrease in intensity which has the determining effect. When he subjected the plane on which the fertilized eggs of FUCUS were situated to partial illumination, it was seen that with the eggs lying on the borderline between light and dark the first partition arose parallel to the shadowborder. The rhizoids come to be on the dark side, and this fixes the direction of polarity which is to govern the entire structure of *Fucus*. Hence the decrease in light intensity, and not the direction of the rays is the determining factor. The underlying cause of this action of light is still unknown, but it is curious that an electric potential gradient also seems to exert some influence.

E. J. LUND [392] succeeded in demonstrating that if fertilized egg-

cells of *Fucus* were placed in an electric field, the first partition is at right angles to the direction of the current. The difference in potential between the two ends of the cell must not exceed $\frac{1}{40}$ volt, to prevent damage. From this LUND concluded that polarity is always determined by electric factors. J. L. MAXWELL's [416] view that light is an electromagnetic oscillation phenomenon might give ground for this conclusion. However, there are objections, as will be seen from the following.

In recent years the problem is fully investigated by D.M. WHITAKER[720] and collaborators. He again studied the so-called group-effect, eggs which lie near each other develop rhizoids on the side toward a neighbour. This effect appears to be the result of increased ion concentration due to the production of carbon dioxide. Polarity also seems to be directed in eggs developing in a gradient of their own diffusion products; the influence of the p_H appears to be very marked. Temperature is effective by changing the acidity due to greater accumulation of the carbon dioxide.

Summarizing we can say that in the development of *Fucus* the axis of polarity can be directed by a variety of external factors: gradients of light, electrical potential, temperature, auxins; in short the idea of C. M. CHILD [111] of more than 25 years ago.

As early as 1885 ED. STRASBURGER [636] observed the inductive action of light on spores of *Equisetum* and in the present century NIENBURG was able to state that in this object the primary effect of light becomes noticeable by the shifting of the chloroplasts. Therefore the cell is then already polar and the first nuclear division is such that the partition arises at right angles to the gradient of light intensity, the same as with the eggs of the Fucaceae. Hence with *Equisetum* also, polarity is induced in the very first stages of development.

What about dorsiventrality, which is likewise a kind of polarity? With regard to *Marchantia polymorpha* E. LEITGEB [370] stated in 1878 that the direction of the first division of the fertilized egg-cell is determined by gravity, though he adduced no conclusive proof for this statement. Moreover, it is difficult to prove because the influence of the surrounding cells of the archegonium is no more to be excluded than that of the embryo sac on the egg-cell of Spermatophyta. The question as to how dorsiventrality arises may be studied better in the bulbils of this species; hence *Marchantia polymorpha* has been the object of this kind of study since 1870. PFEFFER and later W. ZIMMERMANN [756] concluded that in the early stages the bulbils are not yet dorsiventral, though one-sided illumination soon fixes this dorsiventrality in such a manner that the illuminated side becomes the upper side. Once this has occurred, reversion of the dorsiventrality, causing the upper side to become the lower side, is no longer possible in this liverwort, though it is possible with the prothallia of Filices.

During the 20th century this question, which is of importance in con-

nection with the development of the specific form, was repeatedly studied by H. FITTING [178]. His careful investigations revealed that illumination is by no means the only factor to determine dorsiventrality but that gravity is also of influence in the sense that the side which is turned away from the earth becomes the dorsal side. Moreover, the nature of the substrate was found to have a clearly discernible effect. The side towards the substrate becomes the dorsal side, provided that the factors of light and gravity are eliminated as far as possible by placing the object in darkness and on the clinostat.

It is still unknown at what stage the influence of the substrate takes effect whether this occurs after or before germination. Neither the chemical action of the nutrient salts in the substrate, nor a hormone which may be present in the agar (the used material), can be the cause. A gel of pure silicic acid has the same effect.

Another curious fact is the so-called tonic influence of temperature and season on these phenomena. For example, if the lower surface of a bulbil of *Marchantia* is illuminated in winter, that side will become the dorsal side. Thus the stimulus of illumination is stronger than that of gravity. When the same experiment is made in summer, the influence of gravity prevails. This is not merely a matter of temperature, for if the bulbils are heated for some time in winter, the tonus of the winter season remains.

The influence of temperature, or the thermotonus caused by this factor, is also seen from the fact that in germination on an agar-substrate the illuminated side becomes the upper side at a temperature of 18°C, though it becomes the lower side at temperatures over 24°C.

In a later publication FITTING discussed the way in which dorsiventrality is fixed, once this has been induced. Former investigators such as PFEFFER had expressed the view that, in contradistinction to the labile induction in the prothallia of ferns, in *Marchantia* fixation occurs almost immediately, but this is only partially correct. According to FITTING's clinostatic experiments the induced dorsiventrality is still reversible also in *Marchantia*. In entirely ungerminated bulbils induction is established after a geic stimulus lasting two hours or a photic one lasting 5 hours, though it is then still labile. How long this continues to be so depends, where photo-induction is concerned, on the intensity of illumination. For instance, in sunlight the induction in about 85% of the plants will be stabilized after 14 hours. Geo-induction, obtained after 2-5 hours, becomes stabilized after a period about two or three times as long as the induction period.

FITTING considered induction and stabilization as two entirely different processes and compared them to perception and reaction in photo-or geotropism. However, this is a mere hypothesis, unless in this case auxin should play a part.

Be this as it may, with the objects mentioned the contrast between apex and base, upper and lower side, is obtained in the very first stages

of development by induction through external factors like light and gravity, although the property of reacting in this particular way and not otherwise is determined genetically. After a certain time the induced polarity is fixed, so that the present view of this subject agrees with that of SACHS, mentioned above. Later investigations such as those of K. BUSSMANN [100] who studied the induction of dorsiventrality in fern prothallia found it determined by light, gravity and the substrate, the same result obtained by FITTING.

Can the foregoing be regarded as an argument for the theory of A. TH. CZAJA [129] that a stream of auxin, starting from the growing point, polarizes the tissue and its cells? The opposite would at first sight seem more probable viz. that a polar contrast, once arisen, forces the auxin transport in a certain direction. However, by the discovery of auxin many aspects of the problem of polarity have been clarified.

For a discussion of this point we must return to the question as to how polarity reveals itself in the higher plants, the Phanerogams in particular. As stated H. VÖCHTING [684] was the first to subject this question to exhaustive study. He made a number of experiments with cuttings of willow branches and found that at the apical pole vegetative buds and at the basal pole roots developed if a cut branch was suspended in moist air. Polarity is completely fixed and can be influenced by gravity to a limited extent only, as is found when branches are suspended upside down. In that case, though buds do still arise at the apical pole, the roots do not appear exclusively at the basal pole, but also in more apical direction.

It is impossible to mention the great number of such experiments made by different workers with different plants, the only instance I will cite here is that JOH^. WESTERDIJK [714] studying the regeneration of Musci found a very weak polarity in cuttings. Rhizoids were formed at the basal pole and protonema at the apical one, but the location could be readily reversed in cuttings by placing them upside down.

N. JONES [302] succeeded in modifying the polarity of the root of *Crambe maritima* by centrifuging. When non-centrifuged pieces of the roots were placed horizontally, it was found that roots developed from the callus at the apical side and buds at the basal side. But when the roots were for 3 days placed on the centrifuge (1000 revolutions per minute) in such a way that the apical pole was turned towards the centre and the basal pole towards the circumference, bud formation was not limited to the basal pole, but arose also at the apical pole of the root.

As was said above VÖCHTING concluded from his experiments that polarity was an inherent property, and it was not until later on that the investigations of different authors revealed how the direction of polarity may be induced by external factors. But this did not explain the essence of polarity itself, and this point became the more prominent when it was found that there was also a clear polar contrast in the conduction of the stimuli. For CH. DARWIN [132] and later W. ROTHERT [554]

established that in seedlings of Gramineae phototropic stimuli are conducted from the apex to the base of the coleoptiles. We have mentioned this matter in Chapter VIII and described that F. W. WENT [710] after the discovery of auxins showed the polar conduction of the stimulus to be the result of the polar auxin transport from apex to base. As already briefly referred to, H. G. VAN DE WEIJ [716] later investigated this in detail and found that polarity is so strictly that it is even possible for the auxin to be transported from a lower concentration against the gradient to a tissue with higher concentration. VAN DE WEIJ did not venture to give an explanation of this polar character but F. W. WENT suggested that decrease in electric potential might be the cause of the transport of ionized auxin. W. G. CLARK [116], however, found that application of a potential opposite to the inherent one did not affect the polar flow of auxin, and these experiments made the electrical polarity theory untenable. Hence the problem of the polar translocation of auxin is still unsolved.

More or less related to this question is the influence exerted by auxins on the formation of organs. In 1929 F. W. WENT specially studied this influence on the formation of the roots. What led him to do so was an assumption by H. A. VAN DER LEK [371] concerning root-formation. VAN DER LEK assumed that in sprouting buds a hormone was produced which was transported along the base of the branch where in cutlings it stimulated the formation of roots. This author found that in a number of plants there was hardly any root-formation after removal of the buds. Exceptions were those species which already have root germs in the normal branches. In those instances roots grew as strongly from deleaved shoots with leaves. An example of this kind of plants is *Jasminum nudiflorum*. It is only in this case that deviations from the polarity of root-formation may sometimes occur.

In species of the genus *Acalypha* F. W. WENT studied the root-formation of branches with and without leaves. He found that when the branches were cut off immediately after removal of the leaves, root-formation occurred, though to a lesser extent than in shoots with leaves, but when such branches of *Acalypha* were put into the soil a week after removal of the leaves, no root-formation was to be observed. If the leaves are put in water, a substance diffuses from them, which promotes root-formation. If a gel is prepared by partly evaporating the water in which the leaves stood, mixing this with agar it will stimulate root-formation.

E. BOUILLENNE [66] and F. W. WENT in their publication on this subject first termed the substance rhizocaline, but later investigations by THIMANN [649] and WENT showed that this rhizocaline is identical with auxin. The root-formation and the root-forming substance of *Bryophyllun calycinum* was studied by F. A. F. C. WENT.

In connection with root-formation, therefore, we encounter an action of auxins which is quite different from that discussed previously concerning their influence on longitudinal growth and elongation of

different organs in particular. Synthetic auxins, such as indole-acetic acid or naphthyl acetic acid also have this effect on the formation of roots, and commercial horticulture makes frequent use of this fact to propagate hard-to-root plants.

It should be noted that this root-formation is a complicated process, as is shown by the influence of the concentrations of auxin required. F. W. WENT experimented with etiolated seedlings of peas, using the part of the stem situated above the first internode, of which the top had been removed. When a solution of indole-acetic acid was administered to the upper surface, comparatively low concentrations, for instance of $\frac{1}{10^7}$ mole per litre were found capable of causing root-formation at the base of the stem. If the concentration was raised, say to $\frac{1}{10^5}$ mole, roots were found to be formed also at the top, and with a still higher concentration their number would even decrease at the base and increase at the top. Further proof of the complexity of the process of root-formation is found in the fact that yet another kind of substances is active in addition to that of auxin.

(B) Bios factors.

Most interesting of all is the influence of the so-called bios factors on the process of root-formation, but before dealing with this matter it is necessary to devote a few words to the nature of these bios factors and to the bios problem itself.

Although the nature of the problem was still entirely obscure, it first came to the fore in a controversy between J. VON LIEBIG [376] and L. PASTEUR [497] in 1870.

The point of issue then was whether yeast can grow in a nutrient solution containing only pure salts and glucose. PASTEUR said it could, whilst LIEBIG denied this, but the opinion of the former, being the recognized authority on the subject, was generally accepted. About 30 years later E. WILDIERS [730] of Louvain, discovered that yeast inoculated in small quantities in a synthetic substate did not grow. He demonstrated that it did grow, however, when more yeast suspension was used in the inoculation and that the same result was obtained with a larger quantity of malt. Hence the controversy between LIEBIG and PASTEUR arose from the different quantities of yeast used in oculation. LIEBIG was right when little yeast, PASTEUR when much yeast was used.

WILDIERS concluded that yeast needs something more besides glucose and inorganic salts, and that this was present in malt. He termed this factor "bios" and established that it must be of an organic nature, since it becomes inactive when the malt is ashed. He also established that bios resisted boiling with dilute sulphuric acid and could not be extracted by ether or absolute ethylalcohol.

The name "bios" was unfortunate, as it called to mind vitalistic conceptions in which the so-called vital force played an important part, and this was the reason why WILDIERS' discovery was first ignored and later forgotten. It was not until many years later, after the discovery of vitamins, when these as well as the auxins became the centre of interest, that more attention was paid to the "bios" of WILDIERS.

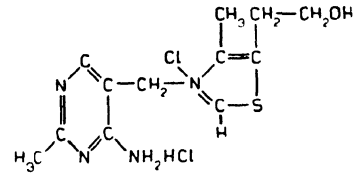


FIG. XXVI. Aneurin

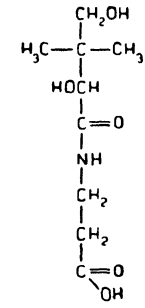
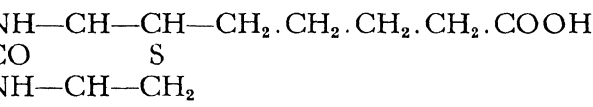


FIG. XXVII. Pantothenic acid

At first the view was held that bios was identical with vitamin B, but this was found to be partially true. Correct insight was obtained when it began to be understood that bios must be a complex. In 1928 E.V. EASTCOTT[152] proved that the sugar meso-inositol was one of these substances (bios I). Later it was established that aneurin or thiamin (vitamin B) was one of its components and in conjunction with pantothenic acid formed bios III.

A third component, bios II, was studied by F. KÖGL [339] and termed biotin. This biotin has the empirical formula $C_{10}H_{16}O_3N_2S$ and according to recent American research its structural formula is the following.



Its presence can be ascertained by the accelerating action which traces of biotin have on the rapidity with which divisions of yeast cells occur.

Higher plants appear to synthesize the components of the bios-complex synthetically, though this is not always the case with the lower plants. Some of the Fungi possess this property, whilst other do not. It has been shown experimentally that *Polyporus adustus*, a species of Polyporaceae, forms biotin but not aneurin, whereas another Fungus, *Nematospora Gossypii* synthesizes aneurin (thiamin) but not biotin. Thus although these two Fungi will grow on a synthetic substrate when combined, their growth is impossible when they are cultivated separately. I may refer also to Chapter VII in which was mentioned that the Hymenocytes which form mycorrhiza generally require aneurin.

As an instance of combined action of the various bios factors an experiment by KÖGL [339] and VAN WAGTENDONK [693] with the pathogenic bacterium *Staphylococcus pyogenes aureus* may be cited here. Growth was measured in % after 48 hours and at 37° C, the bios factors being added in mg. per litre, nicotinic acid and/or aneurin and/or biotin.

nicotinic acid	*aneurin (thiamin*	*biotin*	*growth in %*
5	—	—	10
—	5	—	10
—	—	0.005	140
5	—	0.005	320
—	5	0.005	200
5	5	—	675
0.05	0.05	—	150
0.05	0.05	0.005	665
5	5	0.5	770

It follows from this that the combination is always more active than each component by itself. According to an estimate made by E. C. WASSINK [701], with 0.005 mg. per litre approximately 10^4 molecules are present per cell, a number of the same order as that of auxin.

There are, in fact, yet other bios factors which are still unknown, such as the factor Z which was isolated by H. EULER[163]. W. H. SCHOPFER [583] has studied the action of 2 methyl 1–4 amino-methylpyrimidin which substance according to him is a growth factor of *Mucor* and other micro-organisms.

At the moment the literature on the bios factors of lower organisms has become so large, especially in America, that it is impossible to review them here, but the work of G. W. BEADLE [33] on *Neurospora* should be mentioned before anything else.

There is some reason to assume these hormones to be active in nuclear and cell-division, but be this as it may, the fact remains that biotin strongly enhances the effect of auxin on root-growth. In the experiments referred to the biotin must be brought into contact with the base of the pea-stems, since it is not transported in polar fashion from apex to base, as is auxin, Thus the maximum action of auxin is all but doubled, an average of 18 roots arising per plant instead of 10. It is typical that biotin, when administered by itself, has not the slightest effect on root-growth. This may possibly have some connection with a special stimulating effect on the nuclear divisions which are effective only if associated with cellular divisions and elongation.

In later years the necessity of thiamin, nicotinic acid and vitamin B_6 for root growth has become an important subject, which has especially been studied in America. Many studies of J. BONNER [59], W. J. ROBBINS [546] have been published on this subject, which has been mostly studied with the aid of plant tissue cultures. Excised roots were the material used for the experiments. BONNER, ROBBINS [546] and BARTLEY [31] as well as P.R. WHITE [721] concluded almost simultaneously that aneurin (thiamin) was one of the substances of most importance. BONNER concluded that pea roots require both the thiamin components thiazole and

pyrimidine; some tissues i.e., carrot roots are according to NOBÉCOURT [464] able to synthesize thiamin from the inorganic elements. All plant tissues want an adequate supply of thiamin for normal growth; I may cite here the sentence of P. R. WHITE in the Annual Review of Biochemistry: "Whether or not thiamin must be supplied from without, and what degree of complexity of its constituents is required before these can be utilized by the tissue is a matter which must apparently be decided specially for each tissue. Its function is still a matter of conjecture." ROBBINS c.s. have presented evidence of the importance of vitamin B_6, pyridoxin, for roots of tomato. which conclusion has been verified by BONNER.

(C) Cambium-formation.

Anatomical research reveals that in many cases strong cambium activity precedes root-formation. which fact occasions a discussion of the connection between auxin and cambium growth.

In 1895 L. JOST [305] found that in seedlings of *Phaseolus* as well as in various trees, growth in thickness is dependent upon the presence of leaves though this is not primarily due to their photosynthesis. In this connection JOST referred to a stimulus which is conducted downwards from the leaves without defining this any further.

More than 25 years later CH. COSTER [122] carried out investigations concerning cambial activity and the formation of annual rings in the tropics and concluded that young, growing buds and, to a lesser degree, also leaves produce a hormone which stimulates the cambial activity. In 1933, after the discovery of auxin, R. SNOW [614] succeeded in adducing conclusive evidence of the hormonal nature of this stimulus by demonstrating that it is conducted also through a layer of gelatine. By placing a pea on a de-leaved sunflower seedling he proved, moreover, that this hormone was not specific. SNOW, of course, thought of the action of auxin, though at first he believed that this could cause only extension of the cell-walls, but not cell-division. Later he found that both auxin-a and hetero-auxin can have the same effect on the cambium at a distance of, say 3 cm. below the cut surface. It must further be pointed out that, whereas in various cases cell-division may be caused by high concentrations of these substances, cambial activation is brought about by low concentrations of the same order as may be present in normal tissues.

According to H. SÖDING [615] the process is a matter of secondary auxin action, the auxin activating the cambium which thereupon takes over the further supply of auxin. SNOW drew the same conclusion from the fact that the greater the distance from the place where the auxin had been administered, the stronger differentiation, i.e. the widening of the wood-vessels, became. SÖDING applied high concentrations of auxin, much higher than is required for elongation, and it is possible that this caused differentiation to increase with distance. Such is at least the view of

CORNELIA A. REINDERS-GOUWENTAK [207] and G. HELLINGA [247], who, when applying much lower concentrations of indole-acetic acid established that there was strong growth in thickness also at the cut surface where the auxin was administered. The auxin-effect is lacking if the branches are in deep rest; if however they are forced e.g. by aethylene-chlorhydrine, the effect is quite normal. REINDERS-GOUWENTAK supposes that a wound reaction causes forcing in its neighbourhood. In this manner the auxin-effect can be explained.

The extensive work of E. J. KRAUS [346] and collaborators on the anatomical changes in plants upon administration of auxin can only be mentioned here. A large number of different auxin effects is described in these publications.

(D) Wound Reactions.

Research concerning cell-division at a wound surface was carried out long before anything was known about auxins. In 1921 G. HABERLANDT [222] observed that the less thoroughly cut surfaces of potato and swede were cleaned, the stronger cell-devision at such surfaces occurred, whilst also macerated cells placed upon the cut surface, caused strong division. From this he concluded that at the wound surface the products of cellular decomposition acted as a hormone stimulating cell-division. As the presence of phloem-tissue also promoted division, HABERLANDT spoke of a phloem or leptome hormone, though its chemical nature remained unexplained.

HABERLAND's pupil, B. WEHNELT [706], continued the experiments with a different object, the tissue lining the pods of *Phaseolus*, which offered several advantages. By applying products of autolysis of proteins on this tissue, WEHNELT obtained some result, which led him to suggest that the wound-hormone concerned might be a product of the breakdown of protein.

The discovery of auxins caused these observations to be forgotten, the more so when it was found that auxins can also activate the cambium, as mentioned above. Yet HABERLANDT's observations held out some prospects, because it seemed that in these cases cell-division was caused by substances produced by the protoplast which, though still alive, is in a dying condition. For this reason O. ORSOS [480] continued the experiments with slices of swede-tissue which are more easily kept in aseptic condition. This author found that agar with 1% "Witte peptone" promoted division and elongation, in particular enhancing the production of hypertrophic tissue. Further research showed that the most active fraction was obtained by precipitation with trichloro-acetic acid, which fraction, moreover, yielded a strong reaction with Millon's reagent, which is a reactive to tyrosine. This substance also occasions strongly enhanced cell-division and hypertrophy, though it does not seem to be the active agent in the experiments with *Phaseolus*. In Pasadena

HAAGEN SMIT [218], ENGLISH [158] and BONNER [59] isolated this substance, which was found to be an unsaturated carboxylic acid, termed traumatic acid.

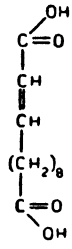


FIG. XXVIII. Traumatic acid

Naturally this does not prove this substance to be active in every wound-reaction, nor does if furnish any proof of this traumatic acid also playing part in the cell-division in normal tissues.

In 1929 F. S. HAMMETT [227] voiced the opinion that a sulph-hydryl combination brought about the mitosis of resting nuclei. HAMMETT stated that:

(1) lead-ions caused inhibition of mitosis in the growing points;

(2) in these growing points lead-ions caused a precipitate with sulphhydryl groups;

(3) in the roots of maize plants enhanced nuclear division may be observed upon addition of a diluted solution of a sulph-hydryl containing substance such as cysteine.

However, all this is not very convincing and one is left with the impression that this is still a vague and obscure problem which may become clarified as our knowledge of the various bios factors increases.

Finally, it should be noted that the theory concerning the action of socalled mitogenetic rays, first expressed by A. GUREWITSCH [216], is pure fantasy, as was shown by the critical investigation carried out by HOLLÄNDER [266] and W. D. CLAUS [117].

(E) Correlations.

In horticultural practice it has long been known that it is possible to cause resting axillary buds to sprout by removing the terminal bud. Sometimes the same result may be achieved by removing the leaf in the axil of which the resting bud is situated. A significant fact is also that plagiotropic sideshoots of the fir-tree will become orthotropic upon removal of the main shoot, and thus ultimately replace this shoot.

J. SACHS was of opinion that organs of similar nature might be regarded as competitors for the acquisition of organ-forming substances and that for this reason the axillary buds had to remain in a resting state as long as the main shoot drew such substances towards itself. It is obvious that this purely theoretical view totally ignored the effect of the removal of the leaves.

As was mentioned in the Chapter I, during the second half of the past century the idea of organ-forming substances found but little favour. It was in M. W. BEIJERINCK's [48] publication on the formation of galls that the idea of growth-enzymes was elaborated further, this author considering that these might be protein substances.

W. PFEFFER spoke of correlative stimulus phenomena ,and HUGO DE VRIES in his Textbook says following: "Hence, in the cases described,

one organ in the course of its normal development exerts an influence upon other parts of the same plant, in consequence whereof these behave in a particular way which changes immediately when they are removed from that influence." Nicely put, though neither of these authors does more than describe facts without in any way enhancing our insight.

K. GOEBEL [202] spoke of "competition", though he was not thinking of organ-forming substances, but of essential nutrients, and in connection with such cases as that of the fir tree, referred above, he used the term "correlative inhibition". In regard to that particular case, it was justly pointed out by L. ERRERA [160] that the plagiotropic side-shoot definitely grows and receives nutrients, so that there must be an additional cause for it to become orthotropic upon amputation of the main shoot.

Before discussing the influence of the leaf upon the development of the bud it should be noted that in the first few decades of the present century the morphologist J. C. SCHOUTE [584] formulated a theory concerning phyllotaxis, which was based on a material influence exerted by the formation of one leaf upon that of another and which theory has a much more physiological character than other theories of phyllotaxis.

The influence of the leaf on the development of the axillary bud was studied more closely by various investigators, of which I will mention only J. LOEB [386], R. DOSTAL [144] and R. SNOW [614]. The significance of polarity in this connection becomes clear, for instance, by the fact that there is root formation at the basal, and bud-formation at the apical pole, as is shown by experiments with cut leaves of *Begonia Rex*, studied by ANNIE M. HARTSEMA [240] and with the well-known object *Bryophyllum calycinum*.

The influence of the buds on differentiation and elongation of the stem of *Asparagus* was observed by OOSTERHUIS [476].

Nowadays, when the idea of totality, i.e. the conception that every individual is one whole in which the functions of the organs are regulated and determined by the whole, plays such an important part in physiology, the general inclination is to view the idea of correlation from this point of view. Yet this by no means implies that correlations are explained by this idea of totality.

In view of what is said above, it is natural, that after the discovery of auxins and bios factors and their importance in root-formation, correlation phenomena should associated therewith.

On the stump of a decapitated shoot SKOOG [608] and THIMANN [649] placed small cubes of agar with the evaporated ether-extract of a culture of *Rhizopus suinus*, which contains hetero-auxin, as mentioned above. This was found to have the same effect as the presence of the terminal bud, namely inhibition of the development of the lateral buds. Later similar tests were made by THIMANN and SKOOG with auxin-a, and this

yielded the same result, though it was found that a rather high concentration of auxin was required, which fact was ascribed to the difficulties connected with the transport of auxins. This requirement of a so much higher concentration was particularly stressed by O. FISCHNICH [177].

These experiments clearly indicate that the presence of auxin or of hetero-auxin must have some connection with the inhibitory effect of the terminal bud on the development of the lateral buds. But what is the nature of this connection and how does it work? The difficulty lies in the fact that whereas in the processes discussed previously growth, whether elongation or differentiation, was promoted by auxin, in this case the contrary seems to happen so that inhibition of growth arises.

Different theories have been formulated in attempts to explain this discrepancy. The first is that inhibition is the result of the direction in which the auxin is translocated. Whereas auxin would have a promoting effect when transported in a basal direction, it would inhibit when transported acropetally. For it stands to reason that in order to reach the lateral buds it must be transported apically in the final stage of the journey. But this hypothesis, which was published by BE. LE FANU [169], does not explain all the facts and is, moreover, at variance with the assumption of a strictly polar auxin transport. proved experimentally by VAN DER WEIJ [716].

F. W. WENT [710] formulated a different theory on the ground of his experiments with seedlings of *Pisum sativum* which had been decapitated and were made to grow in the dark on a solution of sucrose. He considered that besides auxin other substances, such as biotin, were also required for the sprouting of the lateral buds and that it was the function of auxin to direct the transport of those special substances to the locus of auxin production. The flow of these substances to the place of auxin production was the cause of other organs, such as the lateral buds obtaining too little of the substances concerned to be able to develop.

This "diversion theory", as SNOW [614] termed it, seems to imply the old theory of SACHS concerning organ-forming substances and that of GOEBEL [202] regarding the automatic attraction of material for growth towards the place where growth occurs. In the present writer's opinion, the theory does not explain the fact that in lateral buds much less auxin is present than in the terminal bud and that this quantity increases in the former upon removal of the terminal bud. The last-named fact was demonstrated by J. H. G. FERMAN [170], who experimented with *Lupinus albus*, where the auxin content of the inhibiting organs was invariably found to be higher than that of the parts that were inhibited. Hence, says FERMAN, direct action by auxin cannot be the cause of inhibition. On the ground of his experiments this author formulated another theory, which is based on the assumption of SKOOG [608] that the formation of auxin requires a fundamental substance. The existence of this substance, termed "precursor" is supported by a number of facts. The precursor

is transported acropetally, i.e. to the top, mostly to the place where it is converted into auxin. This means that centres for auxin production have a directive action on the transport. Hence dormant buds receive an insufficient quantity of this precursor, so that no growth ensues. But when the terminal bud is removed. the flow of precursor will become directed to the resting buds, which always contain at least a trace of auxin.

This idea FERMAN's is an attractive one, but the problem remains, why is this precursor transported to the place of auxin-production? In order to strengthen his case Ferman quotes from the book "Translocation of Solutes in Plants" by the American physiologist O. T. CURTIS[128], who says that every act which provokes activity of cells in a meristem also stimulates activity of the organs leading thereto. This postulation, which is not an explanation, is another outcome of the conception of the totality of a living organism, just referred to. Furthermore, it remains obscure why addition of a substance such as hetero-auxin, which is entirely different from auxin-a should also stop the precursor transport. But this question is already discussed in Chapter VIII.

Although in his publication on "Growth, Auxins and Tropisms in decapitated Avena Coleoptiles" F. W. WENT ascribes the correlation phenomena to the "free moving auxin" and the phenomena of cell-extension and root-formation to "bound auxin", this does in my opinion not provide a complete explanation. The function and action of auxins are so very complicated, I call to mind the influences on the sprouting of potatoes, the hindrance of the windfall of fruits, that it is impossible as yet to survey them from one point of view.

Finally I mention the interesting case of hyperauxiny in crown gall of tomato, studied by LINK [380] and collaborators. By inoculating with the Schizomycete *Phytomonas tumaefaciens*, a tumor is developed and the experiments give support to the hypothesis that this gall-development is characterised by a disturbed relation of growth substances. The disturbance of auxin relations induced directly or indirectly, by wounding and by the parasite seems to be part of the causal complex of crown gall development. With a higher level of metabolism in the gall is accompanied the fact of a higher auxin content, which gives new support to the view that auxins play some role in the causal complex of normal growth.

(F) Etiolation.

Morphogenesis, or the problem of the way in which the typical form of organisms arises is so complex that an approximation of it is hardly possible as yet. Of course it belongs to the province of morphology, but it also has a genetic, and in regard to development, a physiological aspect. Above a brief discussion was given of the part played in this connection by the idea of correlation as well as by polarity and dorsiventrality, but besides the effect of external factors, particularly

light, on polarity and dorsiventrality, the general formative influence of illumination must now be discussed. Though the light-growth response has already been dealt with, the question as to whether the etiolation phenomena can be altogether explained thereby has not yet been mentioned.

At the time of publication of the 3rd edition of HUGO DE VRIES's Textbook it was known that etiolation was caused solely by lack of light. DE VRIES distinguishes 3 groups of plant-organs according to whether in constant darkness they:

(1) attain the same size and shape as they would when placed in light;
(2) grow taller;
(3) remain smaller.

Apart, of course, from the green colour, the former is the case with flowers and fruits. In the majority of cases the red, blue and yellow colours of flowers are independent of illumination, though the formation of anthocyanin in leaves is definitely influenced by light, being much weaker in the dark. The second is the case with most stems and with stalks of flowers, and also with the ligulate leaves of Monocotyledonous plants.

The third occurs in the leaves of most Dicotyledonous plants, though to a very unequal extent, the leaves of the potato plant, *Solanum tuberosum*, hardly become more than a few mm. in darkness, whereas those of *Phaseolus* or *Beta* attain approximately one-third of their normal size, when kept in the dark.

In those days the cause of etiolation was still entirely unknown. DE VRIES only gave a few views with a slightly teleological slant concerning the question as to which were the organs whose development in the dark might benefit the plant, the idea being implied that the explanation must be looked for in the struggle for existence and the survival of the fittest, postulated by CH. DARWIN.

At the beginning of the present century experiments by D. T. MAC DOUGAL [397] showed that with some plants illumination lasting not more than a few minutes would obviate the typical properties of etiolation. Although there was no formation of chlorophyll, which requires illumination over a much longer period, the form of the leaves was normal, though they remained somewhat smaller. Later G. TRUMPF [660] stated that, as in phototropism, the effect is a function of the amount of lightenergy and the product-rule holds good, a small light-intensity over a long period having the same effect as double that intensity during half the time.

It was in particular G. KLEBS [327] who experimented to ascertain the influence of different kinds of light, and his research may be regarded as being in a sense the continuation of the work of JULIUS SACHS of the previous century. In his interesting experiments with germinating fern-spores, KLEBS found that the normal structure of the prothallium arises

only in strong illumination with sun or artificial light. In weak illumination much elongated cells arise with almost no cross walls. This last-named phenomenon is also clearly observable with red light, but it is a pity that in these experiments, dating from 1907, no mention could yet be made of the quantity of light-energy which produces these phenomena. Moreover, no clear definition is available of the spectral region with which KLEBS worked.

Thus these experiments revealed that there was greater cell-elongation and less differentiation in the dark and in red light, but the problem to be solved is whether the great elongation of etiolated parts should always be explained in this way. Such is indeed, the usual view, but closer examination shows that this is not the only possible explanation in all cases. MAC DOUGAL [397] and later W. BROTHERTON [78] and H. BARTLETT [30] demonstrated that in the dark the cells of *Phaseolus multiflorus* not only become much larger, but that the cell-divisions increase also.

Up till now neither the light-growth response nor the auxin theory has been taken into consideration, though obviously the views of both BLAAUW [51] and F. W. WENT have influenced conceptions regarding etiolation.

The work of V. M. KATUNSKY [312] clearly shows the parallelism between the decrease in growth which may observed after illumination and that of the decrease in the quantity of auxins in this case. If growth in the dark and the quantity of auxins are both assumed to equal 100, it will be found that after strong illumination lasting 2 minutes growth of seedlings of *Avena sativa* amounts to no more than 65% of that in the dark and, that the quantity of auxin is approximately 61%. According to KATUNSKY, after illumination for 16 hours these values are as follows: growth 52%, quantity of auxin 50%, which clearly illustrates the effect of very short illumination of oat coleoptiles, referred to previously. In other cases the difference between the decrease in growth and that of the quantity of auxin may be much greater.

J. VAN OVERBEEK [487] showed that the cell-walls of seedlings of *Raphanus sativus* are much more sensitive to the action of a given quantity of auxin in the dark than in the light. Thus with the higher plants the two facts combined may largely explain the elongation in etiolation although it is questionable whether the difference in the behaviour of Monocotyledonous and Dicotyledonous plants is elucidated thereby. This also applies to the etiolation observed in Fungi. The strong extension of the stalks of some Agaricinae, such as *Coprinus*, which occurs in the dark had already been noted by de VRIES. Here, no auxin is available and the cell-walls are of a quite different nature and structure from those of Phanerogams, therefore the processes must here be totally different. That here in these objects again the blue region of the spectrum has an inhibitory effect was found by various experiments. At first sight this does not agree with the strong positive

light-growth response observed by BLAAUW in the sporangophore of *Phycomyces nitens* when illuminated with blue light.

There is some work by F. W. WENT and collaborators on the spectral sensitivity of stem elongation and leaf growth and the relationships between light and auxin in this respect but it was impossible for me to consult this publication.

Correlation plays a part in the phenomena discussed above. The strong elongation of the internodia of the stem of Dicotyledonous plants is accompanied by a smaller or greater reduction of the leaf surface in the dark, though it is still undecided whether this is only a question of food-competion. The well-known experiments, in which one part of an individual is placed in the light and another part in the dark, yield results in which correlation plays an important part. In the work of J. SACHS on this subject correlation is clearly shown.

(G) Influence of Temperature on Growth.

When DE VRIES's Textbook appeared, plant physiologists maintained the views of SACHS [565], who on the ground of his experiments believed to have found fixed values for the so-called minimum, optimum and maximum of growth, which values varied greatly in different species. HUGO DE VRIES noted that these values lie at a lower level for the germination than for the further development of vegetative parts and that they are higher for species of hot climates than for those of temperate or cold zones. This may either be explained teleologically or by CH. DARWIN's theory concerning the struggle for existence and the survival of the fittest.

Research of the present century has shown, firstly that it is impossible to give absolute values for these three cardinal points and, secondly, that the time factor should also be taken into consideration. From the work done by ELIZA G. C. TALMA [641] in the laboratory of F. A. F. C. WENT [709] it follows that the optimum temperature for growth changes with the duration of that temperature. This fits in with the view expressed by F. F. BLACKMAN [52] that the optimum arises through the co-operation of two factors. Up to about this point the course of growth follows the rule formulated by VAN 'T HOFF [263] and ARRHENIUS [15] with regard to chemical processes, that in the normal zone of temperature each rise in temperature of 10°C causes the velocity of reaction to be doubled, $Q_{10} = 2$. At the optimum the harmful influence of a higher temperature becomes noticeable, such influence being the more harmful, the higher the temperature and the longer its action.

What has been said before concerning limiting factors applies also in the case of growth. A. M. SMITH [612] studied the growth of bamboo in the very humid tropical climate of Peradenya (Ceylon). When he compared the curves of growth by day and night with those of temperature and those of the relative humidity in the same period, he found

that the curve of growth by night was parallel with that of temperature, whilst the curve of growth by day ran parallel with that of the relative humidity. From this it may be concluded that under those circumstances the comparatively low temperature acts as a limiting factor in regard to growth by night, whilst by day the relative humidity of the atmosphere must be the limiting factor. This second point comes clear when it is realized that the lower the moisture, the greater the transpiration and the higher the moisture, the less the transpiration, all other things being equal. When the water supply is limited this may lead to a change in turgescence of the tissues with all the consequences thereof.

Although, therefore, temperature is an important factor in the sprouting and growth of plants, even a dominant factor in regard to the potentiality of existence of plant species in certain climates, the transition from the resting state to the state of activity is not governed by external, but by internal factors. That these autonomic or endonomic factors are governing will be seen presently.

In this chapter we have already given prominence to the idea of correlation and to the part played by auxin in this regard. What follows may, in a sense, also be related to this correlation.

(H) Periodicity of Growth.

This title refers to the phenomenon that growth practically always shows a certain periodicity which usually has some connection with periodic cosmic processes such as the succession of the seasons or that of day and night. Concerning the influence exerted on growth by the dayperiod I would at this juncture say only that this was noted by M. G. STÅLFELT [621] in the nuclear-divisions in the growing points of roots, whilst earlier workers had observed the periodicity of the divisions in the growing point of stems. This occurred both in permanent darkness and in light, though it is uncertain whether the influence of all other factors was eliminated in these experiments.

Reference has already been made in these pages to the yearly periodicity of cambial growth, which HUGO DE VRIES wrongly explained by a difference in tangential tension of the bast in different seasons. As stated, this cambial periodicity was proved to be connected with the auxin produced in the buds and transported in the basal direction. This automatically brings us to the matter of periodicity in bud growth. The question is whether or not there exists a causal link with the cosmic annual periodicity; in other words, whether the periodicity of bud growth is induced or autonomic.

To settle this question, the plants should be placed in an environment where this cosmic periodicity does not exist, but where the length of day, temperature and humidity remain constant, which, if anywhere, may be found in the tropics, below the equator.

Hence the question first began to attract attention when plants could be studied in an even, humid, tropical climate like that of West-

ern Java and the region of the Amazon in South America. Towards the end of the previous century and at the beginning of the present one various botanists had the opportunity of doing so, mostly in „'s Lands Plantentuin", the National Botanical Gardens at Buitenzorg. However, it must be noted that more critical observation does away with much of the illusion concerning the so-called even climate of those regions. For example. Western Java has its dry and its wet monsoon, although in these parts the contrast is not quite so marked as is that in Eastern Java. Notwithstanding this, nearly all the botanists who visited Western Java were struck by the fact that many trees and shrubs there showed periodicity.

According to the observations of J. HUBER [283] in the fairly even and very warm moist climate of Para (Belem) at the mouth of the Amazon, *Hevea brasiliensis*, also shows periodicity. This evergreen tree has bud growth every 1 or 2 months. Later investigations show that such repeated sprouting of buds is by no means rare, but occurs frequently in moist, evenly tropical climates, only a few species having an even, constant growth of shoots. Usually each shoot first develops the lower, transitional leaves, next the foliage leaves, and finally a bud which is enclosed in scales. In species of the type of *Hevea brasiliensis*, however, the various shoots are at different stages of development at the same moment so that the tree as a whole appears to be in full leaf and it is possible to speak only of bud periodicity, though not of annual periodicity.

What HUBER had found in *Hevea brasiliensis*, G. HABERLANDT [222] had also observed in *Palaquium macrophyllum* at Buitenzorg in 1892. HABERLANDT stressed the fact that in this instance periodicity must be entirely independent of external factors and be due to internal, autonomic causes. He found furthermore that trees such as the beech, *Fagus sylvatica*, which in Western Europe are in state of dormancy during the winter, become evergreens when they are planted out in the mountain region of Western Java, e.g. on the Pangerango. Even in that case, however, the beech retains its bud periodicity, as one shoot will be in leaf, whereas another will be in rest and without leaves.

In this connection it may be asked whether repeated bud growth is also found in trees in temperate zones. In a sense this is actually the case, for apart from the normal budding in spring various species have a midsummer foliage, which comes out around the longest day of the year and does so specially vigorously if for some reason or other the normal spring-foliage has failed to develop or has disappeared. In our zones bud growth may occur even in autum, particularly when the summer has been specially warm, as when the horse chestnut, *Aesculus Hippocastanum*, flowers all over again.

In his well-known book, "Pflanzengeographie auf physiologischer Grundlage", of 1898, A. F. W. SCHIMPER [575] viewed the occurrence of rest periods as being an immutable property of plants. G. VOLKENS [685]

and E. SIMON [607], who later visited Buitenzorg, shared that view.

G. A. KLEBS [327] took up an entirely different point of view. (1911) In his theoretical considerations he distinguished three causes, as follows: (1) the special structure; (2) internal conditions; (3) external factors. By the first he denoted what is now termed the genotype, the third term explains itself, but the term internal conditions is less evident. Correlation may be taken to include it more or less.

KLEBS was of opinion that the mutual ratio of mineral and organic nutrients played an important part in bud growth. Whereas an excess of the latter heralded a period of dormancy through inhibition of enzyme activity, an excess of the former causes a renewal of activity. The course of events observed in various species of trees more or less fits in with this idea of KLEBS. In some cased it has, in fact, proved possible to obtain a so-called forcing, a sprouting of resting buds, by providing an increased supply of nutrients salts through removal of the leaves, which causes the total supply of such salts to be diverted to the terminal bud. This will be referred to again in the discussion on forcing.

With *Pithecolobium Saman*, one of the tropical Mimosaceae, KLEBS obtained striking results, though CH. COSTER [122] who later experimented at Buitenzorg with other tropical trees such as *Mangifera indica*, could only partially confirm the results which might have been expected from KLEBS' theory. LAKON [361] considered that the assumption that an excess of organic reserve substances caused or enhanced the period of dormancy, is supported by the fact that the green and the variegated shoots of one and the same specimen behave altogether differently. Variegated shoots of *Acer Negundo*, which, of course, contain fewer organic substances than the green ones, are much easier to force.

Hence KLEBS, by his theory succeeded in shedding new light on certain facts without, however, refuting the conclusions drawn earlier from the behaviour of trees of humid, tropical regions. Summarizing, it may be said that as a result of internal autonomic causes there are several periods of bud growth in one year, though this periodic bud growth, and thus plant growth as a whole, is strictly determined by cosmic circumstances. For instance, the low temperatures which prevail in the spring of our temperate zones inhibit the sprouting of buds which were internally ready to sprout or, in other words, in the late resting stage. Hence, in some cases the connection with external conditions is perfectly clear, though this is not so in the case of tropical trees which show bud growth just towards the end of the dry monsoon as does the so-called "flamboyant", *Poinciana regia*. Perhaps, we may assume in this case the influence of one single shower of rain, as in the case of *Dendrobium crumenatum* which will be dealt with later on.

Now a few words on leaf fall. Generally speaking, the leaf has a certain length of life, the leaves which come out last remain longest on the plant in the autum, as may be observed in the birch. With deciduous

trees the length of life of the leaves in Western Europe is approximately 6 months, but with evergreens like the Conifereae the needles stay on the branch for several years, so that the plant always bears some generations of leaves at the same time. Then the oldest generation falls off, though the others stay on, and the tree is never quite bare. In evergreens like holly, *Ilex Aquifolium*, the leaves have a life time of one or two years.

Leaf fall is an active process, as was known when the Textbook appeared. A dividing layer of cork tissue has to be formed and if this fails to occur as a result, perhaps, of sudden frost at the beginning of the autumn, the leaves will remain on the branch until deep in winter.

Light plays an important part in leaf fall, as may be seen from the fact that many plants drop their leaves after a shorter or longer stay in the dark. During the past few decades it has been found that the length of day is also an important factor. This will be referred to again in the discussion of photoperiodism. At this juncture I will only mention that it is possible to inhibit leaf fall for some time by an artificial lengthening of the day. So there again we find an internal autonomic rhythm which may be modified to some extent by external conditions.

In regard to the problem of annual periodicity, experiments were made particularly with a view to ascertain how growth may be brought about by artificial means, by so-called forcing methods. Horticultural practice had long since used heat to induce plants to flower earlier, but forcing in a scientific way was done for the first time in 1906 by W. J. JOHANNSEN [299], who studied the process with the aid of ether vapour. Later all kinds of means were applied, for instance vapour of ethylene or hydrocyanic acid, warm baths, water injection, wounding etc.

KLEBS considered that it was possible to distinguish natural means, such as a higher temperature or longer illumination, from artificial ones, but this distinction was found to be of little use. KLEBS succeeded in forcing the beech, which was exceedingly hard to force, by subjecting it to constant illumination, which he considered was a natural cause. Later, however, F. WEBER [703] succeeded in forcing the beech by artificial means, by placing it in an atmosphere containing acetylene gas.

It should not be forgotten that this forcing can only be successful at the end of the resting period, when the plant is approaching the termination of its inactivity. At the beginning of the resting period it is as a rule not possible to achieve much by forcing methods, so that here again internal or autonomic factors must play an important part.

That forcing enhances the activity of the enzymes, as postulated by KLEBS's theory, is by no means proved. What, then, is the explanation?

All kinds of hypotheses have been formulated. F. WEBER believed that all means by which forcing was achieved damaged the plant subected to them in some way or other, as a result of which wound-or necro-hormones arose which stimulated cell-division, but this can hardly be proved. More open to experiment, however, is his assumption

that there was a connection between the action of narcotics as a means of forcing and that of a warm bath, seeing that in both cases anaerobic respiration arose as a result of lack of oxygen. This gave rise to experiments by K. BORESCH [60], who demonstrated that the forcing effect of a lukewarm bath on cut branches which are still in their winter rest, was also obtained by a low tension of oxygen during the same period. However, if he caused a high tension of oxygen in warm water, there was no forcing action. This led BORESCH to look for the substances which arise in anaerobic respiration, such as acetaldehyde and ethyl alcohol. These were, in fact, found to be present in catkins of the hazel tree, *Corylus Avellana*, after forcing by means of warm water, but if forcing occurred by means of hydrocyanic acid, their presence could not be definitely ascertained.

The investigations concerning the winter buds of *Hydrocharis morsus Ranae*, must be dealt with in somewhat greater detail. It had long been known that these winter buds germinate only under illumination, whilst if they are left floating in water in the dark, they will keep for years, though they remain in their state of winter rest. In our part of the world they have their rest period, under normal conditions, from the end of September until February, after which germination sets in more or less rapidly according to temperature and illumination. An investigation by E. SIMON [607] showed that red light is particularly effective, whereas blue light has the effect of darkness in this case. This result is rather strange but it is questionable whether sufficient attention has been paid to the intensity of blue light.

A curious point is that weak illumination below the threshold value, which alone fails to cause germination, results in a decrease of sensitivity so that subsequently stronger illumination will be required than in the case of specimens which have previously been in darkness all the time. This might obviously be compared with the tonus in phototropism.

If a practically constant level of temperature is maintained, it is found that sensitivity to illumination undergoes a great change according to the season of the year. As summer draws nearer, the quantity of light-energy required becomes progressively less, and from June until September the influence of light is almost nil. So here again there is an internal, autonomic rhythm, dependent upon the genotype of the plant; this rhythm governing periodicity in the main, though it is influenced and modified by external factors.

According to SIMON, light is an essential factor in germination of *Hydrocharis morsus Ranae*, but this is not altogether correct. AUSEKLIS VEGIS [670] succeeded in causing the winter buds to sprout by subjecting them for a short while to heat at 40°C or for a moment only at 55°C. Calculating the temperature coefficient for this particular instance, VEGIS arrived at such high values as are normally found only with denaturation of proteins. This leads us to the views of F. BOAS [54], who stated that, when colloids grow old, hysteresis, a lessening of

adsorption, may be observed. This results in a liberation of salts which, according to KLEBS's theory, sets the activity of enzymes in motion. In this connection BOAS considered that substances which may serve as a chemical means of forcing lower the surface tension and accumulate at the surface of the protoplasmic colloids, as a result of which other activating substances are liberated. Future research will show whether or not this view contains elements of truth.

Finally the forcing quality of auxin must be discussed. To solve this problem VEGIS studied the winter buds of *Stratiotes aloides*. He found that diluted solutions of hetero-auxin accelerated their sprouting. This was not due to the influence of the p_H ,as with the concentration used by him (1 on 400,000) this does not undergo any change. A remarkable point was the rapidity of the action. Sprouting began 24 hours after treatment, which was totally different from the action of a hot water bath which takes effect only 3 to 4 days later and is, therefore less direct, as argued above.

VEGIS also used *Stratiotes aloides*, the water soldier, to study the action of phosphates on forcing and ascribed his results to activation of the auxin, though this is no more than a gratuitous statement. The difficulty lies in the fact that we do not know exactly how auxin causes activation.

There is one instance in which a greater insight into the problem of sprouting was produced. The phenomenon observed was the opening of the flowerbuds of *Dendrobium crumenatum*, an Orchid of the East Indies. It was observed that on certain days a large number of *Dendrobium* buds all over a district would open, after which a long time might elapse before a new period of flowering set in. This also happens with some other tropical plants. A. A. L. RUTGERS [561] and F. A. F. C. WENT [709] noted that specimens of *Dendrobium*, when removed to Buitenzorg from districts with differing flowering times, came into flower simultaneously. It was clear that the buds had come to a standstill at a certain stage of their development and were waiting for some external stimulus before opening. CH. COSTER [122] succeeded in ascertaining what this stimulus was. He found that a fairly sudden, sharp fall in temperature was required, as will occur in the tropics after heavy rainfall. Nine days afterwards the flowers will then open.

The latter fact clearly indicates that there may be reactions to a change in temperature, and a similar influence of heat is demonstrated by the experiments carried out at Wageningen in the Netherlands under A. H. BLAAUW [51] by a number of investigators, of whom ANNIE M. HARTSEMA [240], IDA C. LUIJTEN [395] and MA. C. VERSLUIJS [677] in particular should be noted.

After careful morphological research and study of the development of the organs the effect of different temperatures on the formation of the reproductive and vegetative parts was ascertained, in both bulbs and woody plants.

The formation and differentiation of flowers and leaves out of bulbs usually occurs in the spring at the stage when, to all appearances, the bulb is in a state of dormancy after the parts above ground have died off. Elongation of the parts then formed does not occur until much later, in the following autumn or early spring. In the trees from the temperate regions formation of the vegetative and reproductive organs likewise occurs generally in the early part of the summer, when the shoots cease to grow and are closed by the bud for the following spring.

The experiments carried out at Wageningen revealed that with bulbous plants the optimum of flower formation lies at a much higher level of temperature than that of leaf formation, though each species has its own specific temperature, that of the tulip being 17—20°C for the reproductive, and below 9°C for the vegetative parts. In the case of the hyacinth these values are approximately 25°C and 13°C respectively. Flower formation of the hyacinth can be induced at any time by a change in temperature during this period, whereas with the tulip a certain number of leaves must have been formed before formation of the flower can begin.

The result of these investigations may be summarized as follows: development is dependent upon the co-operation of hereditary, internal or autonomic qualities with certain external factors and is thus partly autonomic, partly aitionomic. It is the same conclusion we arrived at previously.

In a sense investigations by F. W. WENT [710] concerning the effect of varying temperatures by night and by day agree with this conclusion. Experiments with the tomato under controlled conditions revealed that growth was best when the optimum day temperature of approximately 26°C alternated with a far lower night temperature of 17—20°C. This phenomenon, which WENT termed thermoperiodicity, was ascribed by him to two different processes which he considered prevailed respectively by night and by day, the regulation process during the period of darkness having a lower optimum of temperature than the process which regulates growth by day.

(I) Freezing and Freezing to Death, Cold Resistance.

HUGO DE VRIES graduated in 1870 on a thesis concerning „the influence of temperature on the vital function of plants". This thesis was based on a essay he had written previously in response to a prize offered by the University of Groningen, for which he had been accorded the gold medal. Hence it is understandable that this particular subject was given a fair amount of prominence also in the Textbook. At this juncture we are interested mostly in the action which low temperatures have on plants, in freezing to death. This is a subject which has been studied repeatedly and from entirely different angles during the past 50 years.

When dealing with this subject, DE VRIES discussed the formation

of ice in the tissues, which occurs in the intercellular spaces and results in a withdrawal of water from the surrounding cells. He regarded this lack of water of the protoplasm in the chilled cells as one of the causes of death, whilst pointing out at the same time that a sudden thawing will cause the cells to become flooded with water, thereby damaging the living protoplasm. MÜLLER THURGAU [443] had laid particular stress on the danger of water withdrawal, whereas SACHS regarded the disastrous effect of a quick thaw as the main cause of death.

In his Plant Physiology of 1897 W. PFEFFER [504] pointed out that, according to the former view, freezing to death would be the result of a drying out of the tissues and that it was, therefore, incomprehensible why some species such as *Stellaria media*, the chickweed, do die when they dry out, though not when they are frozen. PFEFFER referred to a resistance to low temperature levels, which varied with the species, but this expression conveys no more than does the statement of the botanist H. R. GOEPPERT [203] of the first half of the past century, that the vitality of each species is overcome by cold at a certain temperature.

At the beginning of the present century an attempt was made by C. MEZ [428] to view the freezing to death of plants from a purely physical standpoint. He compared the cells of freezing plants to a chilling solution of salt, in which the formation of ice is accompanied by the liberation of heat, as a result of which the fall in temperature will be slower than before the formation of ice. With the sudden crystallisation of an undercooled solution the fall in temperature may even give place to a rise. Finally the solution will have become concentrated to such a degree that at that temperature it is saturated and a yet further cooling down of the environment may result in an even crystallization of both water and salt. Under those circumstances the physicist speaks of a cryohydrate of constant composition and of a eutectic mixture. A fall in temperature of the environment will thus result in the continued formation of ice until everything has solidified. MEZ concluded that when the eutectic point had been reached, there could be no more withdrawal of water from the cell-content, so that the cause of death of the cells could not be lack of water. At first this seems a conclusive argument, but it is questionable whether this physical argument is applicable to the living cell. Though there are inorganic salts in the plant cell, there are also sugars and protein colloids, and gradual chilling reveals no trace of a eutectic point, a fact which is no doubt caused by the delayed formation of ice in the small cellular spaces.

N. A. MAXIMOW [415], who repeatedly studied these phenomena, demonstrated with red beet that this object freezes at -2°C and is frozen to death at -3°C, whilst the eutectic point of the pressed juice definitely lies at a lower level than the last named temperature. He reverted to the old view that crystallization of the ice causes the particular damage of the protoplast. In this connection he recalled the fact, mentioned by A. FISCHER [174], that frozen starch no longer possesses its former col-

loid structure and, therefore, its adhesive qualities. Hence he considered that destruction of the structure of the protoplasm colloids was the cause of death.

MAXIMOW further observed that sugars and salts enhanced resistance to freezing to death. After treatment with a solution of glucose, pieces of tissue of red cabbage, *Brassica oleracea s. sp. rubra*, freeze to death at a lower temperature than after treatment with pure water, the mere contact of the boundary of the protoplast with such a solution being sufficient to achieve this result.

It is a well-known fact that young parts of plants, which are rich in protoplasm often withstand freezing fairly well. At first sight this may seem surprising, because these young pearts appear to be so very delicate.

W. STILES [630] has suggested that in such parts of plants, which are proof against frost, the water must be so strongly bound to the hydrocolloids of the protoplasm that it cannot freeze. This assumption for the first time brings us face to face with the contrast between bound and free water.

Among the views of those days SACHS's idea that the death of the protoplast does not occur until the tissues are thawed, became completely forgotten until it was given fresh prominence in a more modern form by the work of W.S. ILJIN [285]. This author regarded the point at which water comes into contact with the dehydrated protoplast during the proces of thawing as the most critical. He considered that if this happened suddenly after previous strong dehydration of the protoplasm, destruction of its micellary structure would result, accompanied by denaturing, coagulation of the colloids. This might provide an explanation of the fact that for many species freezing is more dangerous than a brief period of drying out, and also why the protoplast which has been previously dehydrated runs less risk. ILJIN succeeded in keeping his objects alive, even after they had been chilled to a temperature far below zero, by allowing then only a very gradual supply of water during the thawing process. This happened even in species which normally had little resistance.

ILJIN's work in its turn led W. KESSLER [320] to study the problem of freezing and freezing to death of plants. He wondered whether there might be a causal link between resistance to low temperatures and the osmotic pressure or the p_H of the cell-sap and, if not, whether such resistance might be caused by a change in the condition of the protoplasm.

Experiments with *Saxifraga cordifolia*, *Hedera Helix* and *Sempervivum tectorum* revealed that the resistance of the leaves increases during the autumn, while there is no proportional rise of the osmotic value. This observations, coupled with the fact that during weak narcosis both the p_H and the osmotic value remain constant, whereas resistance becomes strongly diminished, clearly points to the second alternative. KESSLER furthermore gave prominence to the fact that resistance already under-

goes a great change prior to the sprouting of the buds. It has long been known that by reducing the temperature of some seeds it is possible to induce them to germinate more rapidly after a sufficient rise in temperature has set in again. On the other hand we find that in dormancy seeds and spores generally have a very high resistance, as is illustrated by the experiment of the physicist A. C. BECQUEREL [36] who in 1930 chilled spores of the male fern, *Aspidium Filix mas* to a temperature of —270° C without loss of their germinative power.

Resting seeds and spores are characterized by a highly viscous protoplasm, and KESSLER wondered whether this might have some connection with their resistance. His experiments showed that an increase in resistance was accompanied by an increase in viscosity, whereas forcing caused a decrease in viscosity.

Hence there is a definite link between the two, but it remains undecided how the change in viscosity is effected. The most likely view is that this change has some connection with a change of the hydration of the hydrophilic (lyophilic) colloids. The more water is bound and the less water is freely available, the greater the resistance, while in forcing narcotics will cause hydration to decrease and more free water to become available with all the consequences thereof. By calorimetric determination of the quantity of ice in frozen plants S. T. DEXTER [137] demonstrated that in sensitive, less resistant species less bound and more free water is present. However, a sharp distinction between the two kinds of water is difficult to make, an objection which may also be made with regard to the work of B. S. MEYER [426] who studied cold resistance in evergreens.

From 1936 onward the Mc Gill workers G. W. SCARTH [571], D. SIMINOVITCH [606] and J. LEVITT [373] have published a series of studies of hardening changes. SCARTH gives the following summary of resistance.

(1) Intracellular freezing tends to be prevented by increased cell permeability to water, because this accelerates concentration of the cell sap by the growth of ice outside the cells;
(2) Mechanical injury during freezing and thawing with ice extracellular is principally prevented by the reduced structural viscosity of the cytoplasm, or, at least, of its outer zones. Hardy cytoplasm preserves a more fluid or ductile consistency than unhardy when exposed to equal dehydratory force. Further protection is usually afforded by increased osmotic pressure and in very hardy cells by high non-solvent space, both of which reduce shrinkage and distortion of cells.
(3) Dehydration injury at the critical low temperature is prevented by reduced coagulability of the protoplasm-again notably of its ectoplasm;
(4) The protein fraction which is soluble between p_H 5 and 7 is twice as great in *Robinia* bast in summer as in winter.
(5) Increased hydrophily of cytoplasmic proteins indicated by this chemical difference could conceivably account for all the protoplasmic hardening changes.

In the 19th century J. WIESNER has drawn attention to the action of

the fruit juice of *Viscum album*, which inhibits the germination. Many years later, in 1934 A. KÖCKEMANN [338] isolated out of fleshy fruits a substance, the so-called blastocollin which also inhibits the germination of seeds. If germinating wheat seed was treated with this blastocollin, obtained from tomatoes by means of ether extraction, germination remained backward compared to that of seeds not so treated whilst resistance to chilling was enhanced by the treatment. On the other hand, auxin, which induces growth and may serve as a means of forcing, as we saw previously, has a lowering effect on resistance to frost. It is likely that both blastocollin and auxin-a ,or hetero-auxin, act by influencing the ratio between the bound water and the water that is not bound to hydrocolloid, the former substance by increasing the bound water, the latter by reducing it. Is must still be mentioned that this so-called blastocollin is assumed to be a mixture of caffeic acid and ferulic acid.

Thus our insight into this problem of the freezing to death of plants has undoubtedly become enhanced during the past few decades, but it will not be fully understood until our knowledge of the structure of protoplasm as the basis of life has deepened.

(J) Photoperiodism.

It had long been established that some plants do not flower until the autumn, despite the fact that their vegetative development is already far advanced in summer. That the length of day is the main factor in this respect was not understood until two American investigators, W. H. GARNER [192] and H. A. ALLARD [4] drew attention to it in 1920. At our latitude some plants like the soyabean and various cultivated species of aster and *Chrysanthemum indicum* come into flower only when the days are shortening and are not much longer than 12 hours, that is to say in September. To induce them to flower in summer, an artificial shortening of the day is necessary.

On the contrary, species such as wheat, barley, oat flower earlier as the days are longer. Hence flowering may be accelerated by an artificial lengthening of the day in spring. The former species are termed short-day plants, the latter long-day plants, while to the majority length of day is immaterial.

Short-day plants are generally native to those regions where the late summer days are not much longer than 12 hours, hence at low latitudes, whereas long-day plants belong to regions of greater latitude. A curious point is that different varieties of some species vary in this respect, some being short-day plants, others more or less long-day plants. An example of this is the soyabean, *Soja hispida*.

When short-day plants are cultivated in regions where the length of day does not become suitable for the formation of their flowers until late in the season, the vegetative development becomes more strongly pronounced than it is in the regions where they normally occur. The length of illumination may also influence other organs besides leaves and

flowers. There are cases where it governs the formation of the reserve organs below the ground. In the onion, *Allium Cepa*, formation of the bulb is promoted by the long day, and in *Helianthus tuberosus* the short day enhances formation of the tubers.

The effect of the length of day is observable even in the growth of trees. Short-day plants native to regions of lower latitudes, when transmitted to a higher latitude, will form longer shoots which at the end of the season may or may not yet be closed by a terminal bud. Moreover leaf fall is influenced by the length of day, as has been noted on an earlier page; the fall being promoted by a shortening of the days. Lengthening of the day by means of artificial light, however weak, causes the fall to be retarded.

The influence exerted by the length of day during the first stages of development is curious. When subsequently the plants are moved to an environment where different conditions prevail for the length of day, the previous period continues to show its effect. In this case GARNER and ALLARD speak of a photoperiodic aftereffect or induction, a phenomenon which may ultimately lead to a better understanding of this problem that has so far remained obscure.

It is certain that photoperiodism is not directly related to photosynthesis, as is obvious from the fact that it is possible to turn the short-day into a long-day by very weak illumination, for example by candle-light, when the intensity of illumination may remain far below the point of compensation.

Another curious point is that placing a plant in the dark at about the middle of the day has no effect, whereas advancement of the evening or extension of the night does have effect, notwithstanding the fact that in both cases the period of illumination is reduced.

Then there is the question which region of the spectrum is the most important in regard to these photoperiodic phenomena. Experiments reveal that the answer varies with different species. With most species the red region of the spectrum seems to be the important one and to have the same action as daylight, though there are exceptions. From an investigation by G. L. FUNKE [189] it appears that with various Cruciferae blue light acts like daylight and red light has the same effect as darkness.

LYSENKO [396] is the man whose name is connected with the so-called jarowisation or vernalization, i.e. acceleration of the development of plants by certain temperatures to which the seed is exposed. Whereas winter-wheat, sown in the autumn, flowers in its due season, it is unable to form proper ears when sown in spring. In this case that stage of development is reached at a time when the length of day at our latitude is not suitable for flower formation. By treating the seed in a certain way, namely by means of low-temperature treatment, it is possible to cause the formation of ears at the proper moment even when the seed has been sown in spring, hence the term vernalization.

According to LYSENKO, photoperiodism results from the fact that in the

so-called photophase, the second stage of their development some species require definite quantities of light and darkness, as they also have various requirement in regard to temperature at the jarowisation or vernalisation stage. However, this is no explanation but only a description of the phenomena.

An important point is the observation by GARNER and ALLARD that it is sometimes possible to localize the effect of the short or long day. Experimenting with *Cosmos sulfureus*, they exposed the upper part of each specimen to a short day and the centre part to a long day. The result was that the centre part showed strong vegetative development, whilst the upper part of the plant came into flower. Later M. C. CAJLACHJAN [104] continued this kind of experiments with *Chrysanthenum indicum*, a typical short-day plant. The specimens were exposed to long-day illumination, but for a certain number of hours part of the leaves was wrapped in black cloth, with the result that flowers developed in the axillae of the leaves thus partially covered.

These investigations gave rise to closer study of the problem by V. J. RASUMOW [528]. In his experiments this worker used three species of South American tuberous plants, *Ullucus tuberosus*, *Oxalis tuberosus*, and *Solanum demissum*, all of which form tubers only with short-day illumination. Various shoots of one specimen were exposed to short-day, others to long-day illumination. If the number of shoots was small, no tuber-formation arose, though it did occur if the ratio was of 1 to 1, and then in the part that had been exposed to short-day illumination. Investigation as to whether the different parts of one and the same shoot are all equally sensitive revealed that the tip and the growing point are by far the most sensitive and more or less determine the result. Hence, although there is no strict localization, the action is conducted more effectively in basal than in apical direction, which may have some connection with polarity. discussed previously.

The problem is by no means a simple one, and once, again we have to revert to the question as to what determines the development of the reproductive organs, both generative and vegetative, as in the last named case. The question is an old one, dating from the days of JULIUS SACHS, who in "Arbeiten des botanischen Instituts in Würzburg, 1887" published an article on the action of ultra-violet rays on flower formation. In this he expressed the view that there were special flower-forming substances, which were transmitted from the leaves to the vegetation points. SACHS based this view on the fact that several species of plants without reserve organs, when placed in the dark, upon reaching a certain stage of development continue to grow vegetatively, though they do not flower. However, if only part of such a plant like *Tropaeolum majus* was placed in darkness, this did flower. With bulb-plants such as the tulip this is a different matter, because in this instance flower-formation takes place during the dormancy period, as we saw previously.

Furthermore SACHS found that *Tropaeolum*, placed under a jacketed cloche filled with a solution of quinine, did not come into flower either. As he wrongly believed that the action of the solution of quinine in the jacket checked the ultra-violet rays whereas no such checking occurred when the cloche was filled with pure water, he concluded that special flower-forming substances were produced as a result of the action of these ultra-violet rays. These substances, which in his opinion had an enzyme action, would be the cause of flower-formation upon being transported to the growing point. We need not now deal with the reason why this view regarding the action of the solution of quinine was incorrect; suffice it to state that SACHS's theory met with little approval and was soon forgotten.

At the beginning of the present century KLEBS [327] expressed altogether different views. As already mentioned in the discussion on the sprouting of resting buds, KLEBS assumed a contrast between the action of organic and inorganic nutrients and he combined this with the effects of light and temperature. As experimental plant he used *Sempervivum tectorum*, the houseleek, a plant which has an unlimited capacity for continued vegetative growth under strong illumination. However, if illumination is maintained at a high intensive level, while supplies of water and salts are reduced, the rosettes reach a stage where they are ready to come into flower, though externally this is not discernible. This readiness to flower becomes enhanced in the course of spring, which KLEBS attributes exclusively to the low temperature. According to him, readiness to flower is the first stage of flower-formation, the second stage being the formation of the flowerbuds. In its natural state *Sempervivum tectorum* does not reach this stage before spring, but in his experiments KLEBS advanced this stage by means of continuous illumination, that is, he forced the plants. In this respect he considered that the kind of light used was of importance, red light resulting in readiness to flower and to bud-formation, whilst blue light destroyed these. The more intensive the illumination with red light, the more rapid the forcing, though the number of hours of illumination per day also had to be reckoned with. According to KLEBS, this number should not be too small and we might nowadays explain this by referring to long-day illumination. The night has the opposite effect, particularly when temperature is high, a fact which calls to mind the thermoperiodicity of WENT. With low temperatures the stage of readiness to flower may continue for months. In November continuous illumination with 200 metercandles is required for 9 days, in March for only 3 days, and in April for 1 day, hence it may be concluded that in nature this readiness to flower will rise automatically as the days are lengthening in spring.

For the beginning of this century this was a careful analysis of the phenomena exhibited by *Sempervivum tectorum*. The behaviour of other species may show considerable variation. KLEBS pointed out that species like ivy, *Hedera Helix*, and the common speedwell, *Veronica*

Chamaedrys, when exposed to weak illumination, do not reach the flowering stage, but continue to grow vegetatively, whilst under those conditions the last named species develops vegetative shoots but no flower buds in the leaf axillae. On account of the contrast shown by these experiments between the results achieved with red and blue light, of which the former effected a readiness to flower whereas the latter failed to do so, KLEBS believed the cause to lie in the formation or non-formation of products of photosynthesis. This view is no long eraccepted, quite apart from the fact it is questionable whether KLEBS had paid sufficient heed to the intensity of the blue light, not to mention the quantity of light energy. Nor did he take into consideration the length of day, so that further study of this subject in connection with photoperiodism is desirable.

One way of studying this periodicity is the grafting of short-day varieties upon long-day varieties of one and the same species J. KUIJPER [357] and L. K. WIERSUM [726] did this with strains of the soya-plant *Soja hispida*. In this instance, if the short-day variety is grafted on the long-day one, the long-day strain develops flowers, which it would not normally do with a day of $9\frac{1}{2}$ hours, as used in these experiments, though the flowers occur particularly in the vicinity of the graft. It does appear, therefore, that a special flower-forming substance, produced in the short-day plants is transported to the lower part of the stem, where it causes flowerformation. Although evidence was scarce, it seemed that the transport of this assumed flower-forming substance occurred more effectively in basal than in apical direction, as was also indicated by the experiments of RASUMOW [528].

In connection with his experiments, CAJLACHJAN [104], whose work has been mentioned earlier, spoke of a florigene as flower -forming substance, without defining any further what kind of substance this might be. It is highly unlikely that it is an auxin. All this vividly calls to mind SACHS's theory of flower-forming substances, though SACHS entirely ignored the length of day.

The results obtained by K. C. HAMNER [228] and J. BONNER [59] with different strains of *Xanthium pennsylvanicum* are also interesting. This species may be termed a short-day plant in that it still produces flowers when exposed to 15 hours of light and 9 hours of darkness, though it does not do so when the day exposure is longer than 15 hours. When these plants are exposed to short-day illumination the initiation of the flowers becomes microscopically discernible after only 5 days. Exposure of even one single leaf of a specimen to short-day illumination for a period of 20 days proved sufficient to cause the plant to flower. Grafting yielded the same result even in those cases where the graft and the lower part of the stem had not yet knitted together. All this clearly indicates that a florigene is produced in the leaves, provided that the period of illumination is suitable.

An investigation by G. MELCHERS [419] showed that with henbane, *Hyoscyamus*, matters are even more complicated. There are two strains of this species, one being biennial and the other annual. This is due to genetical differences, and the biennial strain is the dominant one and thus prevails over the annual strain in the first hybrid generation. If shoots of the annual strain are grafted on young rosettes of the biennial one, flowering will occur in the same year. Grafting of a leaf also seems to be sufficient, so here again we seem to encounter a transport of a florigene which, apparently, is not specific, since grafting of a shoot of some other Solanaceae, *Petunia* or *Nicotiana*, that has reached the stage of being ready to flower has the same effect. As already stated, the florigene has not so far been identified. It does not seem to be identical with any other known growth-hormone, and according to MELCHERS the instance studied by him indicated a co-operation of the florigene with the vernalin which was supposed to play a part in the phenomenon of vernalization, mentioned on an earlier page.

In the opinion of RODENBURG [551] length of day has two different physiological effects: in short-day plants a certain period of darkness causes the production of florigene and flower primordials, in long-day plants we observe the effect of long periods of illumination, especially of the infra-red part of the spectrum, causing a change in the auxin position with extreme longitudinal growth, the so-called shooting of the flower.

It might be said that research in respect of florigene is at present at approximately the same stage as was that regarding auxins about 25 years ago, when it was proved by P. STARK [622] that placing the tip of coleoptiles of one Graminea on the coleoptile-stump of another species of this family caused the latter to grow in length.

Exactly how such a florigene transforms the zone of growth of a vegetative shoot so as to give rise to flower-formation, is still entirely unknown, but it is obvious that in this case there is an opening for the study of fundamental problems which concern both morphology and physiology.

According to ZIMMERMAN and HITCHCOCK 2.3.5 triiodobenzoic acid induces formation of abnormal flowers in tomato plants.

Chapter XI

TROPISMS AND NASTIES

(A) Classification of the Movements of Organs.

In the Textbook by Hugo de Vries the movements of organs are classified in auxotonic and allassotonic movements. His view was that with the former the movement arose from an increase in turgor tension followed by growth, whereas with the latter alternation of decrease and increase in turgor caused the movement. The second view we are still able to accept, but in the first case we now prefer to speak of growth movements, in view of the fact that growth is not always accompanied by an increase in turgor pressure and that a change in the plasticity of the cell-walls resulting from the action of auxin plays the chief part in growth.

W. Pfeffer, on the other hand, divided the movements of organs into tropisms and nasties, the direction of the former being determined by the external agent, or factor from the outside world, which causes the movement, whereas the direction of the nasties is determined by the internal properties of the organ carrying out the movement. Nasties can be autonomic, or in other words, endonomic movements and the organs carrying out these movements have a more or less dorsiventral structure.

Of the tropisms, phototropism and geotropism have already been dealt with, and of the remainder thigmotropism and chemotropism will be discussed in somewhat greater detail. Chemotropism will lead to a discussion of traumatotropism, hydrotropism, galvanotropism and rheotropism.

Tropisms are with a very few exceptions, for instance phototropism in leaves of *Malva*, growth movements, whereas nasties comprise both growth and allassotonic movements.

(B) Thigmotropism.

This tropism denotes the movements which occur as the result of contact with a solid substance, the direction of the curvature being

determined by the side from which the contact stimulus reaches the organ. When the direction of the movement is determined by the internal properties of the organ, such as its symmetry or dorsiventrality, it is as we saw above, a nasty, in this case thigmonasty. However, it is not always easy to distinguish between thigmotropism and thigmonasty, as will be seen in the discussion on tendrils, where thigmotropism is found in its most typical and most perfect form. This feature of the phenomenon of thigmotropism was studied thoroughly in the previous century. Later it was discovered that several other plant organs are also sensitive to contact. In 1911 this was demonstrated in the case of seedlings by P. C. VAN DER WOLK [739] in the laboratory of F. A. F. C. WENT [709]. Still later the investigations of E. BÜNNING [89] showed that this sensitivity also occurs in petals.

In 1917 P. STARK [622] published the results of an extensive investigation into the thigmotropism of seedlings. This showed that these movements of seedlings fall into two categories which should be clearly distinguished one from the other:

(1) true stimulus movements, based on growth, which are purely thigmotropic;
(2) movements due to one side of the organ becoming limp as a result of loss of turgor through rubbing.

The second phenomenon, which in the classification of DE VRIES might be called an allassotonic movement was observed by J. M. JANSE [295] also in other organs, for instance roots.

The real thigmotropic curvatures, referred to under (1) are entirely different. They also occur upon contact under water and may be induced by one rub with a greased or gelatine-covered stick. With some plants such as the extremely sensitive seedlings of the corn-cockle, *Agrostemma Githago*, STARK observed that growth thus became enhanced by 250%. When the two opposite sides are stimulated, the reaction of that side which was stimulated most strongly will predominate.

In considering tendrils, the organs in which thigmotropism is most strongly developed, emphasis must be laid on the fact that when the Textbook appeared the general view was that contact as such acted as a stimulus. According to DE VRIES, this stimulus was conducted to the opposite side of the tendril, where it increased the turgor pressure. He further maintained that in the first few moments it was possible to make the curvature disappear by placing the just now stimulated tendril in a fairly concentrated solution of salt, though this became speedily impossible owing to the curvature being fixed by growth.

At the beginning of the present century various discoveries were made in this regard, first by H. FITTING [178] and later by G. HABERLANDT [222]. The latter was interested particularly in the way this process is perceived a fact which has been mentioned above. In this connection he laid stress on the tangential tension brought about in the wall by rubbing, a stimulus to which tendrils are exceptionally sensitive. He thought this might have

some connection with the pits which occur in the peripheral wall of the epidermal cells. It was considered that rubbing caused these pits to be compressed, as a result of which the tiny crystal, which is sometimes present in the pits, exerted pressure on the protoplasm, the whole functioning as a kind of tactile organ. One objection to this view is the absence of such pits in extremely sensitive tendrils such as those of the species of the genus *Passiflora*.

Be this as it may, the fact remains that when a tendril of *Passiflora* is rubbed with a match, curvature sets in after 25 seconds, and in the case of *Cyclanthera*, one of the Cucurbitaceae, after not more than 5—7 seconds. The movement may become so rapid as to be discernible with the naked eye. If the stimulus is administered only once, curvature may come to a standstill after only 30 seconds, followed by straightening of the tendril. This movement is sometimes referred to as autotropism, a phenomenon which will be discussed later. Hence, movement is much more rapid here than in geo-or phototropism and it is questionable whether the course of events in thigmotropism can be explained in the same way as that in the tropisms dealt with previously. It is necessary, however, first to discuss the investigations of FITTING [178] who raised various objections against HUGO DE VRIES's assumptions regarding retraction of curvature of tendrils in plasmolysis. FITTING also found that the stimulus engendered by rubbing could be conducted across a piece of tissue that had been narcotized or chilled to a low temperature. The rapid conduction of the stimulus and subsequent response, as well as the exudation of drops from the wound surface recall the course of events in *Mimosa pudica*, which will be discussed later. With these leaf movements, however, the process stops with a change in turgor, whereas with tendrils elongation is rapidly fixed by growth.

Although, on the one hand, it is feasible to assume that the strong elongation consequent upon the contact-stimulus occurs with the aid of enhanced auxin production or translocation, on the other hand there are many objections against this conception. To the present writer's knowledge, no research has been made in this regard.

With *Sicyos* FITTING established that the curvature and subsequent straightening following the administration of one single stimulus is a result of the fact that after the sensitive lower side of the tendril has been touched, first the upper side extends itself and the other side does not begin to elongate itself until the first extension has come to a standstill. Consequently the tendril straightens itself by the so-called autotropism. However, if there is not one, but a whole series of contact stimuli by a support, curvature continues and the support becomes entwined.

It may be that the explanation given by H. E. DOLK [140] for the autotropism in geotropic curvatures also applies to the autotropism in this instance. His explanation is that the first elongation of the side of the organ which later becomes convex uses up more of the material

required for growth than is used at the concave side. When the primary curvature has come to a standstill and the auxin transport is once again equal on both sides, the excess material for growth, which is still available, will cause the concave side to grow more rapidly and thus bring about a straightening of the organ, in this case of the tendril almost automatically.

A phenomenon yet to be explained is the spiral contraction, typical of tendrils, which occurs in a basal direction from the support entwined.

There are tendrils which are irritable on all sides, but those of many species of the Cucurbitaceae are dorsiventral and only react to stimuli from underneath, at least they curve only after contact of their lower surface. However, as FITTING explained, the upper surface is also sensitive to stimuli, though in a curious way, for after simultaneous contact of the upper and lower sides no curvature arises. The stimulus administered to the upper surface can do no more than cancel the effect of the stimulus to the lower surface.

From what has been said, in view of the fact that the direction of curvature of different tendrils for instance those of the Cucurbitaceae is determined by the dorsiventrality of the organ itself, it appears that this is really a case of thigmonasty rather than of thigmotropism.

(C) Chemotropism.

The tentacles of *Drosera*, which were studied by CH. DARWIN [132] in the previous century and described in his "Insectivorous Plants", published in 1874, provide the oldest known example of chemotropism. The phenomena observed here are usually ascribed to chemonasty, although this is not quite correct, as chemotropism also plays a part in them. When the top of a tentacle is touched by a small insect, this tentacle curves chemonastically towards the centre of the leaf. The surrounding tentacles are stimulated also as a result of the stimulus to the first tentacle and curve chemotropically towards the one just touched. This response is not confined to substances of nutritional significance, for it occurs also with a solution of mercuric chloride.

Almost simultaneously with the publication of the 3rd edition of the Textbook by DE VRIES, M. MIYOSHI [432], one of PFEFFER's pupil's carried out an investigation, which may be regarded as very thorough for those days. He studied the chemotropism of all kinds of Fungi, for instance *Mucor*, *Penicillium*, *Aspergillus* and *Saprolegnia*. MIYOSHI injected the leaves of *Tradescantia* (*Rhoeo*) *discolor* with the substance to be examined and sowed spores of the Fungi referred to on the moist epidermis. According to MIYOSHI, under those circumstances the substance injected diffuses outward through the stomata, though it becomes less concentrated. From the fact that the hyphae grow inwards through the stomata, this author concluded that they were positively chemotropic in respect of the substance injected. He also followed PFEFFER's

well-known method and filled small capillaries with solutions of those substances to ascertain whether the hyphae would grow towards them. As a result he found that there was repulsion with a number of substances, for instance with organic acids, alkali, alcohol, potassium sulphate, in various concentrations. On the contrary solutions of ammonium compounds, phosphates, peptones, asparagine and sugars exerted attraction. Glycerol did neither, from which it might be concluded that attraction and nutritional value do not run parallel, as glycerol is a most useful nutrient to these Fungi.

Furthermore, MIYOSHI's experiments showed that the concentration may also be of importance. Sucrose was found to have an attractive effect, increasing up to a concentration of about 14%, but at 50% its action became repelling. According to MASSART [413] this is due to the high osmotic value of this concentrated solution, in other words it is a negative osmotropism.

In many cases the threshold value of attraction is very low. For instance, with *Saprolegnia* it lies at 0.005% for meat extract, and with *Mucor Mucedo* it lies at 0.01% for glucose. In those days no thought was given to the question whether the p_H might play a part in these phenomena.

Apart from Fungi, MIYOSHI also studied pollen-tubes. According to him, these reacted particularly to sugars, whilst there was no action by other substances which do have an attractive effect on Fungi. Later on B. LIDFORSS [375] continued these investigations concerning pollen-tubes and demonstrated that the attracting substance in the stigma of *Narcissus Tazetta* is not a carbohydrate, but a protein substance, a peptone, to which the pollen-tubes of other Monocotyledonous plants reacted also. Nevertheless, owing to its exclusively ecological character the last-named investigation does little to enhance our insight into the problem. Hence H. VON BERG [40] studied germination and growth of the pollen tubes more from a physico-chemical viewpoint. In 1930 he found that the influence exerted by the p_H on germination and longitudinal growth was very great. With a p_H of 5.2 longitudinal growth was 6 to 7 times as large as with a p_H of 3.4 and more than 9 times as large as with a p_H of 8.3, the concentration of sugar being the same in every case. Velocity of curvature of the pollen-tubes differs correspondingly.

With the study of the chemotropism of roots these matters could be entered into more fully than was possible with pollen-tubes. After CH. DARWIN F. C. NEWCOMBE [457] studied this subject, and T. PORODKO [515] in particular investigated the physico-chemical side of the problem.

In his first publications PORODKO described experiments in which he had caused roots to grow in agar, but when these experiments elicited justifiable criticism, particularly from FITTING, he repeated his investigation with an entirely different method with the following requirements.

(1) the chemotropic stimulus had to remain confined to the root tip;
(2) observation should be continuous;
(3) the roots thus stimulated had to be in a humid atmosphere and show hardly any nutations or autonomous curvatures of the tip.

These requirements were met by placing agar cubes containing the solution to be examined against the side of the root tip and microscopically observing its deviation from the vertical. PORODKO found that the effectiveness of the stimulus was proportional with the product of the duration of the stimulus and the concentration, hence the product rule seems to apply. There is a threshold value with just visible reaction. When the stimulus is stronger, the reaction increases to the maximum, after which it decreases, and with very strong stimuli the reaction turns into negative chemotropism. Because of its nature, the borderline between positive and negative chemotropism is hard to determine.

L. JOST [305] regarded many of these phenomena, which PORODKO termed chemotropic reactions, as being traumatotropic ones. It is true that an unbalanced solution of ions may be poisonous to plants. PORODKO's argument for the chemotropic character was that with low and isosmotic concentrations the behaviour of various salts differs greatly, whilst in general non-electrolytes do not cause ,,chemotropic"curvatures in roots.

However, PORODKO's conclusion that the action of a salt is the resultant of the actions of cation and anion, the former having a negative and the latter a positive chemotropic effect, is open to controversy, as are also his further physico-chemical views which cannot be dealt with fully in this context. Moreover, the modern views concerning the significance of auxins to the elongation of the cells had not then been demonstrated.

(D) Traumatotropism.

This concerns the curvatures which occur as a result of a wound-stimulus. As in this process special products, which arise in the dead cells, play the chief part, traumatotropic curvatures might justifiably be regarded as falling within the scope of chemotropism.

The term traumatotropism was used by CH. DARWIN [132] in 1880 to describe the phenomena he observed in roots. When the tip of a root is damaged on one side, whether mechanically or chemically,, the root curves towards the opposite side. This was termed negative traumatotropism by DARWIN.

In 1917 the results of an extensive research in this regard were published by F. STARK [622], who studied traumatotropism in stems of seedlings. If an incision is made on one side of such stems, they will curve towards the side of the wound. STARK terms this positive traumatotropism. The curvature arises not only close to the wound, but also at some distance, and according to STARK the wound-stimulus is conducted both acro-and basipetally. STARK maintained that if he placed the cut

tip of an *Avena*-coleoptile on the stump of another coleoptile of the same species, a wound-stimulus caused by a solution of silver nitrate was conducted across the cut surface.

Since the discovery of auxins and their polar transport these phenomena must obviously be regarded in a different light. As we know, in 1914 A. PAAL [490] expressed the view that in the tip of the oat-coleoptile products are formed, which promote growth and are capable of diffusing across a wound surface. J. BEYER [47] voiced the opinion that interruption of this transport would cause the curvatures referred to. Later this theory was elaborated further by CHRISTINE J. GORTER [206] and N. TENDELOO [646] in the laboratory of F. A. F. C. WENT [709] with special regard to oat-coleoptiles. Whether this explanation holds good in respect of any other cases, particularly roots, is still undecided, and it is also questionable whether all traumatotropic curvatures can thus be accounted for. R. WEIMANN [708] concluded that the explanation of an interrupted auxin transport holds good only for positive, though not for negative curvatures. According to him, negative curvatures are truly traumatotropic and it may be that the wound substances referred to such as traumatic acid have some function in this respect. However, in regard to roots, F. KEEBLE [316] and M. G. NELSON [453] in 1935 accounted for the occurrence of traumatotropic curvatures by ascribing them to disturbances in the distribution of auxin.

(E) Hydrotropism.

Ever since the well-known experiment of SACHS [565], when it was found that roots which were germinating in moist sawdust did not curve downwards in a pure vertical direction, but turned towards the moist sawdust, roots have been said to be positively hydrotropic. The unicellular rhizoids of Hepaticae are also said to be positively hydrotropic, whereas negative hydrotropism is found in the sporangophore of *Phycomyces nitens*.

H. D. HOOKER [270] concluded from his investigations during the beginning of the present century that maximum sensitivity was mainly seated in the root tip, though not exclusively so, as was previously assumed by DARWIN. HOOKER considered that curvature was due to osmotic processes and that it was really more correct to speak of osmotropism. Later H. WALTER [697] demonstrated that with a change in the humidity of the atmosphere the sporangophore of *Phycomyces* shows a growth response. According to this worker, hydrotropic curvatures might arise from unequal reactions on the two sides and hydrotropism might be due to the hydrogrowth response, just as BLAAUW ascribed phototropism to lightgrowth response,

Contrary to this view is the totally different conception of C. E. B. BREMEKAMP [73], who in many cases denies the existence of a special hydrotropism. He argues that it is more correct to speak of hapto-or

thigmotropism, since the essential point in these phenomena is a contact stimulus.

(F) Galvanotropism and Rheotropism.

According to investigations by BRUNCHORST [86], galvanotropism, or the curvatures of some parts of plants, particularly roots, when under the influence of electric currents, resulted from a reaction to the damage caused by the current. The so-called galvanotropism would thus really be a case of traumatotropism. G. GASSNER [193] agreed with this view in general, though he regarded the negative curvatures arising with weak currents as a stimulus-reaction in which the root tip served as perceptive organ.

In contradistinction to these authors, A. E. NAVEZ [451] expressed the opinion that these so-called galvanotropic curvatures were the result of products arising from electrolysis, as fresh roots placed in a solution through which an electric current previously had been conducted also showed curvature. According to this author, the reaction was chemotropic or traumatotropic, which view was confirmed by the fact that when the products of electrolysis were quickly removed no curvature could be observed.

The term rheotropism was used to denote the curvatures of roots against a current of water. At the beginning of the present century F. C. NEWCOMBE [457] devoted an exhaustive study to this tropism and found that the species he examined varied considerably, whilst there were also important differences between specimens of one and the same species. NEWCOMBE considered that not only did the root tip, but als the rest of the growing region react to this stimulus. In his opinion the stems of seedlings showed a positive rheotropism.

Investigations of J. SEN GUPTA [599] in 1929 shed a totally different light on this question. B. HRYNIEWIECKI [281] had demonstrated to what extent the response is influenced by the purity of the water used. SEN GUPTA proceeded to compare the response of roots of *Lupinus* and *Raphanus* seedlings in tapwater with that of roots in twice distilled water. With the former no response could be observed, whereas there was a reaction with the distilled water. But when water was used which was distilled with all the necessary precautions to ensure absolute purity by means of an apparatus such as was first used by FITTING (s. Chapter XII p. 265), no reaction was observed. The same negative result was obtained when the ordinary distilled water was treated with activated carbon. Hence the reaction is caused by traces of poisonous substances which are present in ordinary distilled water. In other words, the so-called rheotropism is in reality a traumatotropism, since the roots are damaged on the side facing the direction of the current. Most likely this, like many other traumatotropic reactions, may be grouped under the heading of chemotropism.

Summarizing, it may be said that both galvanotropism and rheotropism should be regarded as traumatropism or chemotropism and that there is a possibility that the responses are again due to unequal distribution of auxin.

(G) Nastic movements, General Remarks.

We now have to consider those instances where a dorsiventral part of a plant, be it both anatomically and physiologically or only physiologically dorsiventral, makes a movement, the direction of which bears relation only to the position of the part concerned and not to the side from which the stimulus originated. As already mentioned, PFEFFER's term "nastic movements" is generally used in this connection without reference to the mainly autonomous character which DE VRIES previously associated with the term "nasty" in his publication in 1872, when he made a distinction between hypo and epinasty.

In keeping with PFEFFER's conception, moreover, the term nastic movements is used to denote those instances where the movement is caused by growth as well as those where it is due to change in turgescence only. However, there is a fundamental difference between the two, which caused DE VRIES to speak of auxotonic and allassotonic movements. Furthermore, we have seen that in practice there may be some difficulty in drawing the line between tropic and nastic movements, as was shown with tendrils concerning the reaction to contact-stimuli and with the tentacles of *Drosera* regarding that to chemical stimuli.

W. ZIMMERMANN [756] pointed out that the age of an organ is an important factor in deciding which of the terms, nasty or tropism is applicable. As was shown by the foregoing, the dorsiventrality of an organ is determined by external factors and, in its turn, determines the direction of the movement. Nevertheless, it is desirable to follow the accepted usage and to continue the use of the term nasty in cases where an organ executes its movements autonously, without regard to the moment when the dorsiventrality determining the direction of the movement arose.

To begin with, those nastic movements will be discussed which are caused by growth and which can again be separated into autonomous and non-autonomous or aitionomic ones, a distinction which is easier to make theoretically than to carry out in practice. When careful study fails to disclose any external factor as a cause of movement, this is said to be autonomous or endonomous, but it is doubtful if in reality there is not an unknown external factor responsible.

Among the autonomous nasties caused by growth are hyponasty and epinasty which were studied by HUGO DE VRIES in the previous century. With the former, curvature arises owing to stronger growth of the lower side, as may be seen in young fern leaves, the latter being the result of stronger growth of the upper side. Hence the unfolding of young fern leaves is denoted by the term epinasty. HUGO DE VRIES made it clear to

what extent the position of flowers and leaves are determined by hyponasty and epinasty. Later K. GOEBEL [242] devoted a good deal of research to this subject.

As above stated in connection with geotropism it may be concluded from the investigations by H. LUNDEGÅRDH [392], W. ZIMMERMANN [756] and F. RAWITSCHER [531] that neither hyponasty nor epinasty are purely autonomic or endonomic, but that they occur as a result of a dorsiventrality which has been geotropically induced.

(H) Thermonasty.

That a rise in temperature in the environment causes the opening, and a drop in temperature the closing of flowers such as *Crocus* and *Tulipa* is well known, and the same effect is observed with other spring flowers, for instance *Ornithogalum umbellatum, Adonis vernalis*, etc. According to E. J. L. HEINRICHER [246] the species *Dimorphotheca (Calendula) pluvialis* reacts similarly, and GOLDSMITH [204] observed the phenomenon in *Aster Bigelow*. In the case of the Compositae the ray-flowers close.

PFEFFER's studies of thermonasty which date from the previous cent-ury were continued and extended in 1904 by his pupil WIEDERSHEIM [722]. By marks the growth of tulip flowers was measured, both with intact flowers and with specimens in which 5 of the 6 perigone leaves were removed. It was found that removal of the tulip flower from a temperature of 17° C to one of 26° C results in a strong increase of growth of the inside of the perigone leaves, particularly at the base causing opening of the flower After more than an hour this growth comes to a standstill and the outside, which at first hardly gained in length, begins to grow at approximately the same moment when the inside ceases to do so. This causes the flower to close, at least if the first heat-stimulus is not followed by another one.

Experiments with *Crocus sativus* yield the same results with but one difference, for in this instance the increase in growth of the outside sets in at a later moment. To be exact, after its first reaction of increased growth to the rise in temperature the inside discontinues this process to resume it to a lesser extent when the outside begins its period of strong growth, as a result of which the flower closes. Furthermore, *Crocus sativus* reacts to far smaller differences in temperature than the tulip; with a difference of 0.5 °C its reaction is weak.

If a flower petal is tied to ,the flower will nevertheless with a rise in temperature show these two periods of increased growth of the perigone more or less clearly. Thus there is not much reason to assume that the growth of the outside of the perigone leaves in this instance is purely a result of the tension in the tissue caused by the growth of the inside, Contrary to the latter statement made by JOST [305] was the view of WIEDERSHEIM who, like PFEFFER, spoke of two reactions with unequal reaction-times, the outside reacting to the stimulus engendered by the rise in temperature much later than the inside.

A curious point is the reaction caused by a drop in temperature, when the flower closes owing to increase of growth on the outside. This would appear to be at variance with the rule of VAN 'T HOFF according to which growth is supposed to decrease with drop in temperature. However, the explanation is sometimes supposed to lie in the fact that the delayed response of the outside does not occur until then.

There is a curious similarity between this thermonasty of flowers and behaviour of tendrils as a result of contact. The latter phenomenon as described by FITTING was mentioned above and the similarity was the more striking as many tendrils also react thermonastically. This occurs with those tendrils which invariably curve in one certain plane, no matter which side the contact comes from, as well as with those which curve towards the side of contact and thus react thigmotropically. When these tendrils that react apparently thigmotropically are subjected to all sided heating, they will curve in one certain plane and are therefore thermonastic.

The reactions of tendrils and leaves to changes in temperature differ in that flowers open with a rise and close with a drop in temperature, whereas the thermonastic tendril coils up with either. There are also tendrils such as those of *Cissus* which are highly sensitive to contact, yet do not react to heating.

After FITTING had pronounced these views, ZELTNER [753] studied the subject again in 1931. This time, instead of submerging the tendrils in water, as was done before, he experienced with tendrils in humid air and again found a clearly observable reaction to changes in temperature.

During the past few decades many investigators occupied themselves with thermonasty. In 1929 E. BÜNNING [89] concluded that with the tulip a rise in temperature resulted in increased elasticity of the cell-walls. Since he believed that such a reaction was also observable in plasmolysed tissue, BÜNNING considered that movement was the direct result of the action of temperature on the cell-wall and had no connection whatever with the protoplasm. This seemed very strange; nor were BÜNNING's results confirmed by P. P. BÖHNER [56] who declared that they must be due to experimental errors. This author associated the opening and closing of the flowers with a phenomenon which he observed with the tulip. If of a petal the epidermis with adherent layer of parenchyma is removed from the outside and from the inside of another petal, these tissues do not extend to an equal degree when placed in water. With flowers just about to open the inside extends more strongly than the outside, whilst the reverse is the case with flowers in the closing stage. Therefore, the side which is to develop the greater elongation is more apt to absorb water than the other side, though according to BÖHNER it is unable to do so until a change in temperature has set in. In the present writer's opinion this view is open to criticism, nor do the facts support the statement by MA. MÄRKERT [402] that a rise in temperature stimulates the productions of enzyme with local increase

of soluble carbohydrates and enhanced suction pressure. The obvious question is whether auxins play any part in these thermonastic movements.

Finally it should be noted that these thermonastic flowers also have an autonomous nastic movement. During the past few decades it was found that such flowers have an internal, autonomous rhythm of approximately 24 hours. Even with plants reared in constant darkness the flowers open at a certain moment, without any rise in temperature, and this opening is rhythmically repeated. Plants reared in the normal way likewise show this internal rhythm in that much less warmth is required for the opening of the flowers during those periods when the internal rhythm tends to their opening than when this rhythm tends to their closing.

(I) Photonasty.

HUGO DE VRIES has remarked upon the influence exerted by illumination on the position of the rosettes of leaves, pointing out that with strong illumination these assume a horizontal position, owing to epinasty. They seem to press themselves against the soil, whereas they raise themselves when the light-intensity is weak. In this connection DE VRIES spoke of hyponasty and epinasty, but nowadays these movements are termed photonastic, as they are clearly aitiogenic, i.e. induced by external factors, in this case by light.

Much more is known of the photonasty of flowers, which has been subjected to closer study. The species possessing photonastic flowers, flowers which move in response to light and darkness, far outnumber those that are specially sensitive to heat. Moreover, without experiments requiring complicated arrangement it is not easy to decide which is the nasty concerned. Application of heat in the dark is a simple matter, but illumination without heat requires all kinds of precautions; we can even say that it is theoretically impossible. Hence it is very difficult to decide whether besides thermonasty a plant also shows photonasty.

With this photonasty, which may also arise from increased growth of either the inside or the outside of the perianth, the curious phenomenon is to be observed that many flowers have a regular time of opening and of closing. This well known fact led CAROLUS LINNAEUS[381] to construct his so-called flower-clock in the 18 th century. True, these times of opening and closing cannot be determined with exact precision and depend to a great extent upon the weather condition and the intensity of illumination but the rhythm is always present, although, as in thermonasty its only manifestation may be the fact that at certain times the opening requires much weaker illumination and less time than when the tendency to close is predominant.

With many species of plants the opening or closing of the flowers occurs at the beginning of the day or at nightfall, hence the therm nycti-

nastic movements. However, as is demonstrated by the flower clock of LINNAEUS, it is easy to name species whose flowers or flowerheads close before noon, for instance *Tragopogon*, goat's beard, at 11 a.m., or open during the afternoon, for example, the evening primrose, *Oenothera biennis*.

Nyctinastic growth movements also occur in some leaves such as those of different Solanaceae and *Impatiens* species studied by PFEFFER and WIEDERSHEIM [722].

The botanist A. P. DE CANDOLLE [105] was the first to carry out physiological investigations in this regard. As early as 1806 he succeeded in reversing the daily rhythm by means of artificial illumination. Subsequently the subject remained in abeyance for a long time until F. OLTMANNS [473] made the first discovery of importance after the appearance of the Textbook. He found that previous illumination is an essential condition for the subsequent closing of the flower, just as a previous period of darkness is essential for the flower to open completely.

Some years later ROSE STOPPEL [635] carried out experiments with various Compositae such as *Calendula* and *Bellis perennis*, which revealed that these have a periodic movement of the ray flowers resulting in an opening and closing of the flower heads, which is essentially independent of the alternation of day and night. ROSE STOPPEL was of opinion that this rhythm may be greatly affected by an artificial change in the alternation of dark and light periods. Furthermore, her experiments showed that the movement of closing has a strong inducing effect on the re-opening of the flower. Thus a certain measure of agreement exists between this phenomenon and the thermonastic movements studied by WIEDERSHEIM, where similarly an opening movement with a short reaction period is essential to the process of closing with its longer period.

ROSE STOPPEL's best experimental material, the flower heads of *Calendula*, became rigid with constant and continuous illumination, whereas in constant darkness and with a constant level of temperature they carry out a rhythmic movement every 23 or 24 hours. Hence there is a similarity between this and the course of events with the tulip, described in the foregoing section. Opening determines closure which in its turn, determines the next opening movement. In other words, they are relaxation movements, to borrow a term from modern animal physiology. This subject will be reverted to in greater detail in the discussion on the nyctinastic allassotonic movements of leaf joints, where it has been studied more exhaustively by different investigators.

During the past few decades little study has been given to photonasty. TH. J. STOMPS [634] studied the flowers of *Oenothera* and concluded that these open and close in obedience to an autonomous rhythm which was genetically induced.

The events in the night blooming-cactus, *Cereus grandiflorus* were observed by Th. SCHMUCKER [579]. He stated a swelling of the flower bud in broad daylight; hence this nastic movement cannot have been induc-

ed by the stimulus of approaching nightfall. Changes in temperature or of water vapour pressure caused no effect, though the closing coincided approximately with daybreak. Further investigation showed that the first opening, or unfolding occurred about 11 or 12 hours after the beginning of illumination. By keeping the plant in darkness by artificial means, the opening of the flowers could be delayed, but it invariably occurred about 11 or 12 hours after the beginning of illumination.

In other words, the unfolding movement of the night blooming cactus is a greatly delayed after-effect of the stimulus exerted by the transition from darkness to light at daybreak, and in those regions where day and night are approximately equal in length it occurs towards nightfall. With some species of the genus *Cereus* this first transition from night to day continues to have effect for 3 days, the opening then occurring 3 successive times at the same hour of day even with continuous illumination. This after-effect or induced rhythm is independent of temperature or humidity of the atmosphere and is another instance of relaxation movement.

The results of an investigations made by J. D. Vis [683] in the present writer's laboratory and recently published may be briefly summarized as follows: the aitionomic unfolding movement of flowers, which at a certain stage of development occurs only after a light-stimulus, can be distinguished into three types according to the species examined.

Type 1: the unfolding movement is determined entirely by one illumination on the same day (example *Faucaria tigrinum* one of thc Mesembryanthaceae);

Type 2: the unfolding movement is again determined by one illumination which occurred some time before, though a dark period of as long as half a week may intervene (example *Calendula arvensis*);

Type 3: the unfolding movement occurs only after 2 light stimuli with an interval of 24 hours (example *Tragopogon pratensis*).

The secondary movements which follow the unfolding movement occur in fewer species. When Calendula is kept in constant darkness, a series of secondary flower movements follows, the rhythm of which has been determined autonomously, though external stimuli may vary the moment of maximum opening. *Tragopogon* in constant darkness yields only one reaction, again with a rhythm of 24 hours. To all appearances *Faucaria* shows no autonomy though there is a rhythm in its sensitivity to stimuli, similar to what was said previously concerning the thermonasty of tulip flowers.

But, however interesting all this may be, it does not materially enhance our insight into the fundamentals of the metabolism of these organs, which must, after all, create the conditions leading to the opening and closing of the flowers. Because this is still obscure, attention must be drawn at this juncture to the investigations by van Herk [253],

which were referred to in the chapter on respiration and which form a first step into this unknown territory of physiology.

Studying the chemical processes in the spadix of *Sauromatum guttatum*, VAN HERK ascertained what it was that caused the sudden rise in respiratory intensity which in turn, leads to the rise in temperature in the spadix. The latter phenomenon plays a part in the flowering as much as does the nastic movement of the spatha of *Sauromatum*. His investigation showed that this rise requires sufficiently strong illumination of the inflorescence at least 20 to 22 hours beforehand. Furthermore it was found that the action of an activator is essential, this being a substance which is produced exclusively in that part of the inflorescence which bears male flowers. Under normal conditions the transport of this activator to the spadix where the intensive respiration is due to begin, occurs about 21 hours before the rise in temperature sets in, though it is possible to bring about this rise in temperature by injecting an extract of the male flowers into the marrow of the spadix. The nature of this activator, which in a sense is similar to the florigene referred to in connection with photoperiodism, is still unknown. However, VAN HERK found that this substance in unstable and is already used up before the rise in temperature sets in. The action of this activator in the process of metabolism is also still obscure. The fact that the reaction takes a long time makes it seem unlikely that we should think of any direct participation by the activator in the respiratory processes, nor is it probable that the general activity of the tissue is enhanced. A more probable assumption is that the substance concerned should be regarded as a pro-activator.

(J) Allassotonic movements, General Remarks.

Contrary to the movements discussed up till now, these allassotonic ones arise as a result of changes in turgor, they are reversible and largely confined to leaves with leaf joints, the pulvini. In the majority of cases they occur in compound leaves such as those of the Leguminosae, Oxalidaceae and the Pteridophyte *Marsilia*: only in a rare instance are they found in simple leaves such as those of the Marantaceae.

In the joints, the vascular bundles are found in the centre and rigidity depends upon the turgor pressure in the parenchymatous cells of the thick cortical layer. The obvious conclusion is to associate changes in the position of the leaf with changes in the turgor pressure of the upper and lower sides of the joints. This is the conclusion drawn in HUGO DE VRIES's Textbook of 1895, but though it appears to be so simple, this matter has given rise to a great deal of controversy, as will be seen presently.

Allassotonic movements may be divided into:

(1) endonomous or autonomous movements, the classic example of which is the leaf of *Desmodium gyrans*, one of the Papilionaceae. This

species has ternate leaves, the small side-leaflets of which circumnutate. Under favourable conditions of moisture and temperature the little leaflets execute jerking movements in such a way as to cause the tip of the leaves to describe a complete circle or ellipse within a few minutes. DUTROCHET was the first in stating the autonomous character of these rhythmic movements. Afterwards they were studied by W. PFEFFER and in the beginning of this century by H. MOLISCH [436] and by K. HOSSEUS [277]. The latter showed that these circumnutations are greatly enhanced by a rise in temperature, the optimum temperature being approximately 30° C. Different Oxalidaceae also make such endonomous movements.
(2) aitiogenic movements, resulting from external factors. These may be subdivided into nyctinastic and seismonastic movements, of which the latter are the result of a jerk or shock, the former having some connection with the alternation of day and night. It was object of much study how close this connection is and whether these movements should be regarded as being more or less autonomous.

(K) Nyctinastic, Allassotonic Movements.

Despite the work done by PFEFFER, WIEDERSHEIM [722] and LEPESCHKIN [342], concerning the movements of pulvini during the alternation of day and night, the mechanism of this movement was still so little elucidated that in the Textbook written by L. JOST [305] and BENECKE [38] in 1923 it was referred to as an unsolved problem. It was elucidated later as a result of investigations by W. ZIMMERMANN [756]. Experimenting with *Phaseolus* and other Papilionaceae, this worker found by means of incipient plasmolysis that the osmotic value at the upper side of the joint increases by night, whereas it decreases by day, the course of events at the lower side being the reverse. This is accompanied by periodic swelling of one of the two sides, which gives rise to an erect position in the morning and a declining position in the evening. Hence the view of HUGO DE VRIES was found to be mainly correct.

Much more study has been devoted to the problem of the cause of the nyctinastic movement than to that of its mechanics. It must be stressed that leaves response to light and darkness by means of their joints, an observation made in the days of ancient Rome.

PLINY [512] in his book "Historia naturalis" stated that plants assumed a sleeping position at the beginning of a total eclipse of the sun. Hence such species are photonastic, though it is still undecided whether some internal, autonomous factor is also involved in the movement.

In his Textbook DE VRIES did not go deeply into this problem, nor did he take sides in the controversy between J. SACHS and W. PFEFFER [504]. According to the former. the alternation of night and day did no more than regulate a periodic movement which was dependent upon internal causes and thus an autonyctinastic movement. the latter, on the other

hand, in his earliest publications on the subject viewed the nyctinastic movement as purely paratonic or aitiogenetic, i.e. as the result of external factors, chiefly the alternating intensity of illumination, hence as purely photonastic.

In any case ,with *Phaseolus, Canavallia ensiformis* and some other Leguminosae the problem is a more complicated one, for if one of these plants is placed upside down the movement of its leaves is inverse and apparently governed by gravity. A. FISCHER [174], who subjected these plants to closer investigation in 1890, termed such species as *Phaseolus* geo-nyctitropic, but it would be also possible to call them geo-nyctinastic. However, when plants such as *Trifolium pratense, Acacia lophanta* and some others are placed upside down their leaves execute their movements in a direction determined by the new position of the plant hence they are auto-nyctinastic.

Although originally PFEFFER stressed the aitiogenetic character of these movements, he later changed his mind as a result of a publication by R. SEMON [598] and came nearer to SACHS's point of view. For by this assumption of "Mneme" SEMON tried to argue the view that the plant inherited a certain rhythm from its ancestors.

An entirely different conception is found in the publications by ROSE STOPPEL [635] of the years 1916–1926. In *Phaseolus* seedlings reared in constant darkness, this author observed normal nyctinastic movements. It might be thought that this could be accounted for by SEMON's "Mneme", but this was refuted by experiments with seeds from South America, which after germination executed their movements in accordance with the rhythm of the country where they were raised and not with that of the country of origin.

Hence ROSE STOPPEL concluded that an external factor was involved in the nyctinastic movements, some factor which coincided with the alternation of day and night, though our senses were unable to perceive it. According to this author, it could not be the alternation of night and day itself, for, as previously mentioned in the discussion on the nyctinastic movements of the flower heads of *Calendula*, these movements continue even in constant darkness. ROSE STOPPEL believed that the daily fluctuations in electrical conductivity of the atmosphere might constitute a factor of this kind which is not perceptible to our senses. If this assumption was correct, the movements should cease when plants were placed under isolating covers, and this was precisely the result she believed to have obtained.

As ROSE STOPPEL's experiments in this regard were not very convincing they were repeated with better methods and varying results by E. SCHWEIDLER [595] and A. SPERLICH [617] also by H. CREMER [125]. CREMER demonstrated in 1923 that neither an isolated position nor enhanced ionization of the air had the slightest effect on the nyctinastic movements for the curve representing the conductivity of the atmosphere is by no means parallel to that of the leaf movements. On the other hand, it

was CREMER's belief that the emanation from radio-active preparations did have effect. That emanation, the gas formed by radium and thorium is itself radio-active and precipitates in particular on wires that are electrically charged. Hence there was the possibility that at Basel, where ROSE STOPPEL conducted her experiments, this emanation was stronger than at Würzburg, where CREMER worked and that it had precipitated on the isolating cages used by ROSE STOPPEL. Thus the precipitation of this emanation might be the cause of the different results of their respective experiments.

However, the matter became even more fantastic by CREMER's experiments in a very deep salt mine in the Harz mountains. For, whereas in the salt mine itself the plants reared there showed no nyctinastic movements at all, upon being transported to the earth surface they were found capable of acting. *Phaseolus* plants, reared on the earth surface under constant illumination, continued to make nyctinastic movements, provided that their joints were wrapped in black cottonwool. But when they were transported into the salt mine their nyctinastic movements ceased and were not resumed when the plants were returned to the earth surface. This gave rise to the idea that the movements required the action of rays which were unable to penetrate into the deep mine.

Subsequently, investigations were made by G. BROUWER [79], who worked with the tropical Papilionacea *Canavallia ensiformis* in the laboratory of G. VAN ITERSON [288] at Delft. The physiological behaviour of this species differs from that of *Phaseolus* in that it continues its nyctinastic movements when reared under constant illumination, though with less regularity. Since BROUWER was of the opinion that a specimen of *Canavallia*, in which artificial illumination has induced a different rhythm of movement, loses this rhythm when placed in an environment with constant illumination and returns to its original day and night rhythm, he, too, assumed that an external factor was the cause of the leaf movements. However, ANTHONIA KLEINHOONTE [329], who worked in the same laboratory and used the same experimental plant, *Canavallia ensiformis*, arrived at entirely different results. She found that when *Canavallia* is reared under absolutely constant conditions of temperature and illumination, it executes irregular and non-synchronous movements. The synchronous movements observed in BROUWER's experiments were caused by minute fluctuations in temperature, amounting to no more than some tenths of a degree. When plants whose rhythm has been advanced 12 hours by subjecting them to a few minute's illumination for a couple of nights, are placed either in constant light or in constant darkness together with other plants which still possess their normal rhythm, it will be found that each of these two groups retains its own rhythm for some time, provided that the temperature is kept absolutely constant. Under identical circumstances one plant will then be in the night position, whilst the other is in the

day position, which clearly shows that the rhythm is *not governed* by external circumstances or factors.

Hence ANTONIA KLEINHOONTE reverted to Sachs's old view regarding an autonomous rhythm, though without being able to define its causes or its action. One of the strongest arguments for this assumption is the fact, already noted by PFEFFER, that the duration of the period is not always 24 hours, but sometimes 22 or 23 hours. Hence it cannot possibly be some factor which returns with the alternation of day and night that determines the movement. According to K. STERN [627] and E. BÜNNING [89] the period depends upon the temperature in that it becomes shorter as the temperature rises, so that at 35°C it is only 19 hours, whereas at 15°C it is as long as 30 hours. Without the cosmic factor of the alternation of day and night, the respective rhythm of the different plants would not be synchronous as its length is different. This view is practically identical with that held by SACHS in 1863, i.e. the movement is autonyctinastic though photonastically governed by the alternation of day and night.

There remained the question whether it was possible to form some idea of the way in which this natural and endonomous rhythm arises and is maintained even in constant darkness or light. G. J. DE GROOT [214] made an attempt to solve this question in the thesis he wrote in the laboratory of V. J. KONINGSBERGER [343]. The starting-point for his observations was the structure of the joints of *Phaseolus multiflorus* and *Canavallia ensiformis*, as well as the way in which the day and night movement occurs. As was mentioned above changes in the volume of the upper and lower cortical tissue are the cause of the movement. According to W. ZIMMERMANN [756] these changes are dependent upon charges in the osmotic value; previously, H. WEIDLICH [707] had arrived at the same conclusion, but his method was criticized by E. BÜNNING [89].

With the aid of a simpler method DE GROOT was able fully to confirm ZIMMERMANN's conclusions. In the day position the osmotic value of the cortex at the upper side of the pulvinus is at its minimum and that at the lower side is at its maximum, whilst the reverse is found in the night position.

It is known that also in the joints there is a starch-sheath on the border between the cortical tissue and the central cylinder. Normally this starch sheath contains a great deal of starch. When the plant is placed in darkness, where the nyctinastic movements of this species gradually become less intensive and finally come to a standstill, this starch gradually decreases and has practically disappeared by the time the movements cease. This might be taken as an indication that starch plays a part in these processes in that it supplies the energy required for the movement. DE GROOT tried to trace the connection by arguing as follows. The action of amylase will cause the starch to be converted into soluble carbohydrates, sugars, which enhance

the osmotic value. Hence, if there is a difference in the quantity of amylase, this would provide the explanation. However, quantitative determination in the endodermis meets with technical difficulties and, moreover, the activity of this enzyme depends to a large extent upon the p_H. Therefore, DE GROOT suggested that a more or less rapid conversion of the starch may be due to rhythmic changes in the p_H. This is possible, as the value of the p_H in the joint fluctuates between 5.2 and 6.5, and the activity of the amylase undergoes considerable changes between these two values.

DE GROOT offered the following explanation for the mechanism of the movement in the dark. In respiration sugars are consumed or diffuse to loci with lower concentration, whilst the sugar concentration may be maintained by the action of amylase. If the last process prevails, so that the sugar content increases, the osmotic value will rise and water will be attracted with the result that the volume of the cells and thus of the whole of the tissue is increased. The electrolyte content will then automatically decrease and it seems reasonable to assume that this will cause a decrease of the concentration of hydrogen ions in the solution, which is not buffered. Thus the p_H is changed in the sense that the action of amylase in inhibited, which is bound to lead to a decrease in the sugars. In this way a certain periodicity would be obtained and the movement would thus be a relaxation movement, one process creating the conditions for the other, and vice versa. Although a great deal of DE GROOT's theory is still entirely a matter of conjecture, it should be considered as first attempt to analyse the rhythmics of the movement.

However, in the laboratory of BUDER [88] it was observed that the osmotic concentration in the joints, taken totally, remains the same. The periodic swelling would be caused by formation, not of sugars as DE GROOT asserted but of still unknown electrolytes.

(L) Seismonastic, allassotonic movements, Conduction of Stimuli.

These movements which occur after contact producing a shock, are found in varied organs of higher plants such as stamens, pistils and pulvini or leaf joints, They have been studied mostly in *Mimosa pudica*, the sensitive plant. This Leguminosa, which is native to South America, though it is now a common weed in the tropics, has double-jointed leaves, with leaf joints of the 1st, 2nd and 3rd order. When one leaf is stimulated, this droops first, gradually followed by the other leaves, and with a strong wound-stimulus such as that of scorching, other leaves in the vicinity may also be seen to droop. Hence it is clear that there must be conduction of the stimulus. The most favourable temperature for this conduction lies between 25 and 30°C and with temperatures below 15°C conduction is no longer observable.

In regard to the mechanism of the movement, LINDSAY [378] in 1790

was one of the first to experiment by cutting away the upper side of the joint. As a result of this operation the leaf drooped, but it soon recovered and practically resumed its former position. It retained its irritability, which led LINDSAY to conclude that the upper side of the joint is only of subordinate importance. More than 30 years later DUTROCHET [149] arrived at almost the same conclusion, and it was not until 1848 that new observations were added by E. H. BRÜCKE [85]. This worker set himself the task of solving the problem as to whether intact joints are equally flexible before and after stimulation. He found that if the plant was placed in an inverse position, flexibility of the joint was two or three times greater after stimulation than before. Hence it follows that stimulation causes the turgor to decrease, from which it is evident that the mechanism of the seismonastic movement differs fundamentally from that of the nystinastic movement where flexibility was found to be less at night.

Twenty-five years later W. PFEFFER succeeded in confirming BRÜCKE's observation and in demonstrating experimentally that the upper side increases, whilst the lower side decreases in volume. What does actually occur when the joint is stimulated? LINDSAY had noted that the colour of the lower side became darker after stimulation, even if movement was rendered impossible by fixing the leaf. The dark colour strongly resembled that of a tissue which had been injected with water into the intercellular spaces. PFEFFER cut the leafstalk at the joint with a sharp knife and found that if the cut leaves were placed in a space saturated with water vapour they recovered their susceptibility to stimulation after some time. If they were then subjected to fresh stimulation, a drop of moisture was seen to appear on the cut surface, which obviously originated from the intercellular spaces. Moreover, when this exudation had dried up, PFEFFER observed a crystalline residue, from which he concluded that other substances besides water must have entered the intercellular spaces or in other words, that permeability of the boundary layer of the protoplasm in the cortical tissue of the joint had become strongly enhanced as a result of the seismonastic stimulation. In other words exosmosis results from the process of seismonastic stimulation.

For a long time these observations of PFEFFER regarding *Mimosa pudica* remained unconfirmed, but an investigation of BÜNNING concerning irritable stamens, which will be discussed later in greater detail, proved that in that case also permeability is strongly enhanced by seismonastic stimulation.

In his Textbook HUGO DE VRIES merely mentions the exudation from the cells without expressing himself on the matter of increased permeability, though he describes the course of the process as outlined above.

In a sense the question of the conduction of the stimulus is far more interesting than that of the mechanics of the movement, and this problem has repeatedly formed an object of study.

As early as 1837, DUTROCHET the great plant physiologist of the first half of the 19th century, made ringing experiments with *Mimosa pudica* in order to find the route along which conduction occurred. He assumed that in his experiments he had removed the tissues as far inward as the xylem, and from the fact that the stimulus was conducted across the wound he concluded that conduction took place along the xylem. According to him, this conduction was due to a difference in hydrostatic pressure.

After DUTROCHET, PFEFFER was the first to raise the question again. He held the same view as DUTROCHET, though he laid stress on the fact that a stimulus reaction following an incision in the stalk was invariably accompanied by exudation originated from the wood vessels. The fact that it was possible to demonstrate conduction along a narcotized part of the stalk led PFEFFER to reject the assumption that conduction might occur along living cells.

The next worker to deal with this subject was HABERLANDT [222], whose book "Das reizleitende System der Sinnpflanze" (The conducting system of the sensitive plant) of 1896 contained discussion on the problem. He observed conduction of the stimulus along a dead piece of stalk 10 mm. long, whilst later D. MAC. DOUGAL [397] found the same with a piece 30 mm. long, thus supporting the view that the stimulus conduction was of a hydrostatic nature and occurred exclusively along the wood.

Nevertheless, objections were raised. For although HABERLANDT observed this conduction along a dead piece of stalk, he also saw that the exudation, which appears on the cut surface upon stimulation following an incision, originates from tiers of cells, situated one above the other in the cortex. The cells contain protoplasm and are the homologues of the rows of cells which contain tannin and are found in other Leguminosae. It is curious that when P. J. F. MEYEN [424] made the same statement in 1839, it failed to make any impression.

Haberlandt assumed that these tiers of cells of *Mimosa pudica* had pores in their transverse walls, which made it possible for the liquid to move about,but he expressed no opinion on the contradiction between this conduction of the stimulus along living cells and the conduction found to occur along a dead piece of stalk.

For this reason, however, CUNNINGHAM [127] criticized HABERLANDT's work, as the cells could no longer be turgescent after the leafstalk and the joints were killed by steam. A further objection to HABERLANDT's assumption was the stimulus conduction in the genus *Neptunia*, a Leguminosa which lacks the living cells referred to.

Next, the exactness of DUTROCHET's ringing experiments was called in question, and this led K. LINSBAUER [382] to repeat these with special precautions. His results were the same, provided that a violent stimulus was administered, such as submersion in boiling water.

Thus there were two diametrically opposed views, the one holding

that conduction occurred along living cells in the cortex, the other that there was hydrostatic conduction along the xylem. The adherents of the former view alleged that the stimulus constituted a local increase in pressure in those cells, which was propagated by the elasticity of the walls of the turgescent cells, and that it was this undulatory movement jerking the joint which caused the reaction.

Such was the state of affairs when in 1916 U. RICCA [543] published his sensational experiments with *Mimosa Spegazzini*. First of all *Ricca* opposed the view of those physiologists who believed that the stimulus was purely a question of hydrostatics. HABERLANDT had assumed that if leaves were scorched with a burning match, which causes intense stimulation ,water vapour was formed, which would increase the tension. According to RICCA this could not possibly be correct for the following reasons:

(1) the stimulus may be applied by contact with water at 70°C;
(2) a cut leaf may be stimulated again if the cut surface is renewed after it has recovered.

As the hydrostatic channels, which might serve in conduction are then open at one side, there can be no question of any increase in pressure. Moreover, Mac Dougal observed no reaction if the pressure with which water was pressed into cut stalks was raised suddenly to 8 atmospheres. RICCA continued the experiments concerning stimulus conduction along pieces of stalk killed by heating beyond 100°C and found that conduction occurred along a dead piece 5 cm. long. He also observed the following curious feature. He bisected a stem under water and reconnected the cut ends through a glass tube filled with water. Then, after removal from the water, it was possible to stimulate the basal part by burning to such an extent that the parts above the glass tube reacted. In other words, the stimulus could be conducted through a glass tube filled with water. A manometer attached to the tube revealed no trace of any change in pressure, but from the lower cut surface a greenish solution was seen to appear, which moved through the glass tube to the top part, whereupon reaction set in. According to RICCA, those substances were carried along with the transpiration stream, hence the stronger the transpiration, the more rapid the conduction of the stimulus. If the lower cut surface was sealed, no water transport occurred, nor was there any stimulus conduction. Finally, RICCA prepared an extract from the tissue of *Mimosa Spegazzini*, in which he placed twigs which had fully recovered from their cutting. After a while, when part of the extract had been transported to the joints by the transpiration stream, a clearly discernible reaction set in.

These most convincing arguments made a great impression in botanical circles, and at various places experiments were made to test the view that a substance, which RICCA regarded as a hormone, was responsible for the reaction of the leaf joints. In central Europe the re-

sults were mostly negative, but R. SNOW [614], who repeated the two experiments with *Mimosa pudica* in Trinidad, obtained positive results, both with cut twigs placed in extract and with the glass tube experiment. According to RICCA, boiling the extract in the former experiment did not destroy the active principle, but SNOW found that it did.

H. FITTING [178], experimenting in Bonn, confirmed RICCA's results and tried to discover what the stimulating substance really was, but failed to get any definite results. In 1936 A. SOLTYS [616] and K. UMRATH [665] extracted the stimulating substance from *Neptunia*, whereupon it was purified and concentrated to such an extent that it retained its activity even when diluted to 1 in 10^8. However, the purified preparations lost their activity after a couple of days, and the chemical nature of the substance is still unknown.

In order to ascertain whether living cells are at all involved in the stimulus conduction, FITTING chilled part of a leafstalk to 2°C, but this did not retard the conduction. This and the fact that conduction could occur along a narcotized piece, were grounds for rejecting the assumption of co-operation by living cells in the process of conduction.

However, the observations by J. C. BOSE [63] were at variance with this conclusion. This worker as early as 1914 started experiments in which plants were electrically stimulated. Under such circumstances no conduction was found to occur along a zone chilled to 2°C. In regard also to another aspect remarkable results were obtained by this investigator from India. He found that if weak electric currents were used stimulation occurred only when the circuit was closed and the joint functioned as cathode. With higher voltages, approximately 12 volts, stimulation occurred not only when the circuit was closed and the joint acted as cathode, but also when the circuit was broken and the joint acted as anode. A curious point is that all this shows almost complete agreement with what is observed in animal nerves that are electrically stimulated. This will be reconsidered later; suffice it for now to state that BOSE referred to a conduction along living cells similar to that in animal nerves. According to him, another argument for this view is the fact that the velocity of conduction is greatly dependent upon temperature, the Q_{10}, being about 2.

In regard to the velocity of conduction, K. LINSBAUER [382] found that if *Mimosa pudica* was touched with a hot platinum needle, velocity amounted to 5-9 mm. per second. With stimulation by incision, velocity was greater, amounting to approximately 30 mm., and with complete severance it amounted to 130 mm. per second or 7,8 M. per minute. SNOW [614] found more or less the same values and wondered whether a transport along the wood vessels could, in fact, be responsible for this, seeing that the maximum velocity of the transpiration current (in tropical lianes), according to CH. COSTER [122] does not exceed 80-250 cm. per minute. SNOW also observed other instances

where stimulus conduction was so abnormally rapid that he referred to "high speed conduction". For example, an incision made into a shoot as far the cambium causes the leaf to droop almost immediately. The same result was obtained by N. G. BALL [27] with shoots under water, where a negative pressure as high as it is generally assumed to be in the transpiration stream seems most unlikely. BALL spoke of "rapid conduction".

At the time of these investigations, about 1925, the idea began to gain ground that *Mimosa* must have more than one kind of conduction system. This might explain the conflicting observations and opinions of the various workers. It was thought that the system with slow conduction might be that of a transport of the stimulating substance, as yet unknown, along the wood, whilst the other, more rapid system passed along living elements.

It was also about this time that K. UMRATH [665] began his important research into this subject. What follows is partly derived from his observations. The phenomenon of the reactions of living organisms to stimuli must now be reviewed somewhat more broadly.

A living organism, a living system, is never in equilibrium. Absorbing external energy it develops into an unstable physico-chemical system ,in which potential differences arise. By stimulation some form of additional energy is imparted, which tends to equilibrate the system and thus provides the reaction to the stimulus. In some instances there is a clear proportionality between the energy supplied and the reaction, for example in phototropism. Sometimes it is a case of an "all or none reaction", in other words a certain quantity of energy has to be imparted for the reaction to occur completely and with full intensity, while application of more energy fails to enhance the reaction and less energy provokes no reaction at all. Such "all or none reactions" are found in *Mimosa* and in plants with irritable stamens.

After a reaction of this kind the unstable system is destroyed and has to be reestablished, which requires time and energy. Until this recovery has been effected, reaction is found to be impossible and the plant is in what is termed a "refractory stage". As stated, recovery occurs at the expense of energy, that is by means of a respiratory process, for without a supply of oxygen no recovery is possible.

Characteristic of such stimulation phenomena is the arising of a so-called action current with a change of electric potential. For example, if an internodium of *Nitella*, a genus of the Characeae is stimulated, whether by mechanical, electrical or chemical means an electric negativation sets in after about 0.1 second, which culminates after one or two seconds. The stimulated internodium cell is approximately 100 millivolts more negative then a cell not so stimulated. Subsequently the reaction declines, negativity becomes less, and after 5 to 10 minutes the resting potential is once more obtained, as was shown by UMRATH'S investigations. With this particular plant the action current lasts a few

seconds only, and the absolute refractory stage from 5 to 10 minutes.

Hence this action current may serve as a criterion of the arising of a process of stimulation, even if the plant shows no external symptoms. Cases where external symptoms may be observed, as with the leaf joints of *Mimosa* or *Neptunia*, or the leaves of *Dionaea muscipula*, or the irritable stamens and pistils. are exceptional, With these the action current also arises and although its culminating-point may vary between 10 and 100 millivolts and the period of the refractory stage may vary likewise, the essence of process is the same.

A similar process of stimulation is found in the animal nerve, where an action current and a refractory stage also arise, from which it follows that the nerve likewise has to pass through a process of restitution before being capable of fresh activity. However, with the nerve the entire process is much more rapid, as will be seen from the following.

Objects	*Time of rise of action current*	*Velocity of stimulus conduction*
Internodium cell *Nitella*. . . .	1.2 sec.	2.3 cm. per sec.
Primary leafstalk *Mimosa pudica*	0.6 sec.	2.5 cm. per sec.
Stamen *Berberis*	0.1 sec.	—
Leaf *Dionaea muscipula*	0.2 sec.	20 cm. per sec.
Nerve *Anodonta* (mussel) . . .	0.1 sec.	4.6 cm. per sec.
Mantle-nerve *Octopus* (cuttlefish)	0.01 sec.	300 cm. per sec.
Nerve Vertebrate.	0.0002 sec.	10,000 cm. per sec.

The culminating point of the action current, the change in potential, is in the nerve even less than in the plant cell. The period of the refractory stage is also different and where the time of rise of the action current is short, the refractory stage is also of short duration.

Objects	*Period absolute refractory stage*	*Period relative refractory stage*
Internodium cell-*Nitella* . . .	4—40 sec.	60—150 sec.
Stamen *Sparmannia*	30—60 sec.	500—1000 sec.
Leaf *Dionaea muscipula* . . .	0.6 sec.	30 sec.
Rectal nerve frog	0.05 sec.	—
Rapid nerve Vertebrate. . . .	0.0005 sec.	0.001—0.01 sec.

With the bladders of *Utricularia* the process of opening and closing takes less than 0.1 second, hence conduction is probably even more rapid than with *Dionaea muscipula*. With the action current the equilibrating process does not require the presence of oxygen and is probably not a true chemical reaction, but a physical change of the colloids of the

boundary layer of the protoplast. However, there is also some ground for comparing this process to an instance of wounding. For in that event there is also a change in potential which causes the so-called wound-potential to arise and, moreover, wounding is frequently accompanied by a distinct loss of semi-permeability. In other words, permeability is strongly enhanced, which leads to the assumption that this abnormal enhancement of permeability is at least in part responsible for the wound potential. By analogy it might be assumed that the action current is likewise a case of enhanced permeability.

The maintenance of the so-called resting potential of the living cell requires unequal permeability of the membrane to cations and anions. The former permeate out of the cell more rapidly than the latter, which causes the outer layer to become positive in respect of the interior of the cell. If the wounding permeability is strongly enhanced and the boundary layer becomes more permeable to anions, the surge of potential will be more or less levelled, causing an electric current to go to the place of wounding. If an explanation of the action current is sought in the assumption that enhancement of permeability cancels the resting potential, the magnitude of the action current must be dependent upon the level of the resting potential. This has been found to be the case in various instances.

To revert to the restitution of the irritable state, this begins as soon as negativity has reached its culminating point and requires the presence of oxygen, as already stated. Hence it is a respiratory process.

With the aid of the highly sensitive method of using a thermo-couple it was found that in *Mimosa pudica* a rise in temperature resulted from the intensified respiration during this period. However, this rise in temperature may have some connection with the action of movement in which formation of organic acids was observed as a result of oxidation processes.

Various theories were propounded to explain conduction of the stimulus in which the obvious similarity to the conduction in animal nerve has to be taken into account. According to the physical theory, the action current functions as an electric stimulus, whilst the chemical theory regards as the principal point the formation of a special stimulating substance which is assumed to propagate itself along the surface of the protoplasm more or less after the manner of an ignited fuse.

As was stated previously, FITTING discovered the presence of a stimulating substance in *Mimosa pudica*; SOLTYS [616] and UMRATH [665], later isolated this substance out of *Neptunia*, but this product obviously does not play any part in the process under discussion. Theirs was probably the substance which is translocated by the water current through the wood vessels, but we are concerned here with a substance moving in living cells along which the electric current also passes, i.e. the rapid conduction in contradistinction to the slow conduction through the wood.

With reference to *Mimosa pudica* the investigations carried out by A. L. HOUWINK [278] in the laboratory at Utrecht should be noted. If HOUWINK stimulated *Mimosa pudica* by contact with a drop of water below 10° C, he found there was conduction of this stimulus along the living cells, accompanied by a change in potential, the action current already referred to. He stated furthermore that this conduction could occur through the stems, the primary and secondary leafstalks and the joints, though sometimes it was interrupted at the secondary joints. Conduction depended upon temperature as observed by BOSE [63] and did *not* pass a zone chilled to below 5° C. Hence this is the conduction investigated by BOSE and termed the „rapid conduction" by BALL [27].

In addition a second process of conduction may be observed with wound stimuli, for example by burning, which may occur both through living cells and by a transport of stimulating substance arising from the wounding. This is the conduction specially studied by RICCA [543], which passes along a dead zone and in which the substance in the wood vessels is transported by means of the transpiration current. According to HOUWINK this again is accompanied by a change in potential.

Finally, there is probably yet a third kind of conduction which occurs when leaves are cut off. In that case the stimulus is conducted in the ways described and, in addition, there is a specially rapid conduction in the principal leafstalk, particularly in the case of young leaves and in a very moist atmosphere. This stimulus can pass along a chilled, though not along a dead zone, and this is the conduction which SNOW [614] termed the „high speed conduction".

Summarizing, it may be said that great progress has been made in the analysis of the phenoma exhibited by *Mimosa pudica*. It has been found that the course of events is considerably more complicated than it was thought to be and that the modern view of the existence of processes of conduction along totally different routes and in totally different ways explains the earlier conflicting observations.

After this extensive discussion of the seismonasty and the stimulus conduction observed in *Mimosa*, it is possible to be brief on the subject of the processes occurring in irritable stamens and pistils, studied in particular by E. BÜNNING [89] during the past few years.

These processes have already been referred to in general. Here, again, stimulation by contact leads to a sudden enhancement of permeability, as a result of which the content of the vacuole is largely taken up by the cell-wall which expands strongly in consequence of the intake of water. Later the solution appears even on the outside of the cell-wall. The stamens of *Sparmannia africana* are particularly suitable for the study of the process. As a result of exosmosis the cells are shortened, and this causes the sudden movement of the stamens. According to BÜNNING, the stimulus is propagated gradually along the successive epidermal cells and this would cause the peculiar, jerking movement registered. However, the correctness of this view can be called in quest-

ion for, if so, the number of jerks should be much greater. It is likewise uncertain whether the change in permeability is due to a change in the state of coagulation of the protoplasm, though it was observed that the anions of the HOFMEISTER [264] series diminish susceptibility to the reaction in the same sequence in which they augment the hydration of the hydrophilic (lyophilic) colloids.

As already stated, after reaction these stamens also have a refractory stage when restitution occurs only in the presence of oxygen, but the details of this process are still unknown. Whereas BÜNNING invariably observed that there was first a latent period and that reaction did not set in until a short time had elapsed, M. J. DIJKMAN [138] who worked in the laboratory of F. A. F. C. WENT [709], found that with powerful stimuli reaction set in practically immediately, at least within the first second. This must be due to a momentary and all but complete loss of the semi-permeability of the boundary layer of the protoplast.

Hardly any study has been devoted to the movements of the pistil, for example in *Mimulus*, when this is subjected to contact by jerks, but the irritable stamens of some Compositae such as *Centaurea Cyanus*, the corn flower, and other species of Cynareae have been investigated. However, the study of this subject has not progressed far beyond the stage reached during the previous century. It is obvious that in this case there is considerable contraction of the filaments of the syngenesious stamens, for observation with the naked eye shows that the stigmata folded together are pushed through the tube of the contracting filaments so that the pollen appears. According to K. LINSBAUER [382], contraction sets in within a second from contact and ceases after 7—13 seconds. During the next 50–60 seconds, the refractory period, the original length is resumed, whereupon the stamens are irritable once again.

During this movement the filaments of *Centaurea* undergo considerable contraction, up to about 10–30% of their original length, and in this process water enters the intercellular spaces. The same thing occurs with other irritable stamens. Hence the course of the process is likely to be the same as that described in connection with the investigations of BÜNNING and of DIJKMAN concerning *Sparmannia*. Curious also is the plasticity and elasticity of these filaments of *Centaurea*, which upon being relaxed by plasmolysis may be stretched to double their length without this extension being necessarily permanent.

Reference has been made to the stimulus conduction in *Dionaea muscipula*. There the two halves of the leaf close together when the hairs situated on top of the leaf are touched. Various views have been expressed regarding the mechanism of this movement, which in 1875 was investigated by CH. DARWIN [132]. He concluded that the layers of cells above the midrib actively contracted by about 6% when the leaf closed. Leaves killed by being placed in boiling water do not close, and in narcosis closure is very slow, hence it must be a vital process. This view remained generally current until 1916 when W. H. BROWN [82]

expressed an entirely different opinion. Instead of a contraction of the upper side he assumed a sudden expansion of the cells on the lower side of the midrib. He believed this expansion to be the result of a rapid extension which was later fixed by growth. According to BROWN, the increase in size following the first extension is much greater than that after the next, but this hardly tallies with the fact that the velocity and intensity of the closing movement is practically the same. Nor are this author's conclusions very acceptable in other respects. The problem was, therefore, subjected to further study by another physiologist, H. VON GUTTENBERG[217]. His views were again opposed by E. ZIEGENSPECK[754], but it would be beyond the scope of this book to enter into the details of these controversies. It remained undecided whether with *Dionaea* it was a question of growth, the movement being similar in its velocity and quickness to that of the tendrils, or whether it was more like that of the leaf joints and thus of an allassotonic character. However, in view of the general remarks made above, it is more likely to be a movement resembling that of leaf joints.

In recent years the subject is studied by ASANA [16], but I was not able to consult his publication.

Undoubtedly the labellum of the flower of the Orchid *Catasetum* provides another instance of a process of stimulation. Upon contact, the viscid disc of the rostellum is suddenly torn loose, the stipes becomes extended and scatters the pollinia. This also occurs when the two antennae are touched by a gentle stroke and it cannot possibly be a purely mechanical transmission of tension, but must be a process of stimulation. Hence VON GUTTENBERG includes the phenomenon among the seismonastic movements and, in fact, some of the other Orchids also have a labellum susceptible to stimulation.

Finally I will briefly touch upon the well-known movement of fruits with a so-called explosion mechanism such as *Ecballium elaterum* and *Cyclanthera explodens*, where, upon contact, the juicy, ripe fruit suddenly bursts asunder. Formerly it was believed that with these Cucurbitaceae it was not a case of stimulation, but of the turgor tension gradually mounting or the solidity of the cell-walls decreasing, to such an extent that finally the tissue was no longer sufficiently coherent to resist the tension and the smallest shock from outside was enough to cause the tissue to tear apart at its weakest point. However, VON GUTTENBERG sought to show that this is also a case of stimulus action and that contact suddenly raises the turgor tension; similarly to what occurs in *Dionaea*, resulting in a tearing apart of the tissue of these fleshy fruits. It is not yet known whether this explanation also holds good for other fruits which explode upon contact, such as those of *Impatiens noli tangere*.

OVERBECK [486] studied the explosion mechanism of *Ecballium elaterum* especially in regard to the turgor value.

CHAPTER XII

LOCOMOTOR MOVEMENTS, FINAL OBSERVATIONS

(A) Amoeboid Movements.

The term locomotor movements is used to denote those active movements by which organisms or certain organs are capable of moving bodily from one place to another. This phenomenon is encountered, on the one hand, in organisms which are not surrounded by a cell-wall, and on the other hand in micro-organisms which possess a cell-wall. In higher organisms they are particularly found at those stages of development where these organisms are, in a sense, comparable to micro-organisms. The movements in organisms without a cell-wall are termed amoeboid movements, whilst in those with cell-walls active motion by means of cilia or flagella is customarily denoted by the term taxis or tactic movement.

One of the most curious vital phenomena is the fact that the protoplasm is in motion. The naked protoplast is capable of making amoeboid movements, in which not only the surface may extend in one place and retract in another, but in which there are also internal movements in the protoplasm. Sometimes it seems as if inside the surrounding, quiescent protoplasm there are channels through which the moving protoplasm is flowing, but that is only an illusion. The flowing protoplasm keeps on widening and narrowing as was observed by L. RHUMBLER [542] and H. S. JENNINGS [297]. From this it might be concluded that the so-called circulation and rotation in the walled cells agree with these amoeboid movements in the naked protoplast, and this assumption is supported by the fact that protoplasmic currents can occur in plasmolysed cells.

At the end of the last century G. BERTHOLD [45] and, after him, O. BÜTSCHLI [90] tried to gain deeper insight into the nature of these movements. They did so by imitating them as far as possible with inanimate material, the surface tension of which was changed locally. Just how this was done is at present immaterial for our purpose. According to them, the amoeboid movement was due to successive unequal surface tensions in the living protoplasm, resulting from certain metabolic processes. RHUMBLER later referred to a gelation of the surface, but this again leaves the nature of the process unexplained.

Later it was pointed out that an electric charge lowers the surface tension and that in the amoeboid movement the boundary layer may

possibly become charged. In an electric field amoebae go to the cathode, whereas bacteria go to the anode. If we assume a bacterium to be a negatively charged particle sending out hydrogen ions, it follows that these will lessen the surface tension of the amoeba which is positively charged and pseudopods will be projected. If the bacterium is enclosed, as a result of which the opposite charges may neutralize each other and the surface tension of the amoeba is raised, the pseudopods containing the bacterium will be retracted. The positive charges of the amoeba was ascribed to the secretion of carbon dioxide, though no explanation was given of the negative charge of the bacteria.

Hence the nature of the amoeboid movement is far from clear and this becomes even more obvious if the chemotactic movements of the white blood corpuscles or leucocytes are taken into consideration. For these also carry out amoeboid movements, and a certain kind of leucocyte behaves as follows:

Negatively chemotactic	*Indifferent*	*Positively chemotactic*
10% sodium salt	distilled water	1% papayotine
0.5% solution of quinine	1% sol. of peptone	living bacilli
0.1% lactic acid	1% sol. of antipyrin	dead bacilli

Experiments by H. J. HAMBURGER [226] showed that the calcium ion has a specific action as it stimulates the phagocytes, whereas magnesium ions do not do so. Traces of substances which dissolve fat and lower the surface tension enhance phagocytosis. Presently the discussion on the chemotactic movements of spermatozoids and gametes will show the complexity of the phenomena of attraction and repulsion. Perhaps the colloid-chemical views of BUNGENBERG DE JONG, mentioned Chapter III will be able to explain amoeboid movements.

(B) Protoplasmic Streaming.

As stated in the previous section, protoplasmic streaming in walled cells closely resembles the amoeboid movements. For research purposes it offers certain advantages over the amoeboid movement, hence it repeatedly constituted an object of study.

Firstly the influence of temperature was studied, and it was found by B. CORTI [121], who discovered protoplasmic streaming in CHARA in 1774, that there was a minimum at 2°C and maximum at approximately 40°C. His observation was forgotten and in 1807 TREVIRANUS[658] rediscovered it but first the studies of G. B. AMICI [8] aroused attention and gave rise to the discovery of the streaming in other objects, *Vallisneria* and *Hydrocharis* by MEYEN [424], *Tradescantia virginica* by ROBERT BROWN [81].

More than 25 years later DUTROCHET [149] established the minimum at -1°C, the optimum at approximately 35°C, and the maximum at 42°C. He still termed it movement of the cell-sap, and it was not until HUGO VON MOHL's [435] discovery that the term protoplasmic streaming began to be used.

Another 50 years later, in 1876, W. VELTEN [671] studied the velocity of streaming in the leaves of *Vallisneria* and found that up to 31°C this velocity gradually increased, whilst at 41°C rigor occurred. The rate of protoplasmic streaming varies with the species as well as with the conditions. In slime molds the velocity surpasses that in walled cells: VOUK [686] records 1.25 mm. per sec. in slime molds, EWART [165] reports 2-3 mm. per min. for CHARA, whilst for *Vallisneria* and *Elodea* values of 1.5 mm. are given.

About the beginning of the present century A. J. EWART [165] concluded that the increase in velocity with a rise in temperature was due to a decrease in viscosity. F. G. WEBER [703] vainly attempted to prove this conclusion by means of the drop-method, the shifting of particles in the streaming protoplasm under the influence of gravity or centrifugal force.

Hence the problem was by no means solved when HILLE RIS LAMBERS [258] began to devote his attention to it in the laboratory of F. A. F. C. WENT [709]. He found that between temperatures of 15°C and 30°C the increase in the speed of the flow is directly proportional to the rise in temperature. With *Nitella*, the plant used by him, this proportionality held to 40°C if heating was applied only for a very short while. HILLE RIS LAMBERS ascribed this phenomenon also to changes in viscosity, though he adduced no strict proof. Though the rigor which sets in at about 40°C, is at first reversible, it ceases to be so.

HILLE RIS LAMBERS attributed the rigor to the following causes:

(1) withdrawal of the free water from the disperse phase of the colloid protoplasm;
(2) withdrawal of water from the hydrated particles of the protoplasm. Hence withdrawal of both bound and free water.

Investigations by G. ROMIJN [550], likewise in the Utrecht Laboratory, followed a few years later. This worker paid special attention to the effect of rapid changes of temperature on the protoplasmic streaming of Characeae. Like HILLE RIS LAMBERS, ROMIJN found that a rapid rise in temperature did not lead to cessation of the streaming, though this did come to a standstill with a rapid drop, provided that this drop exceeded a critical value. According to ROMIJN, the smaller the range required the more sensitive the protoplasm. This is the case at approximately 20°C, when a drop in temperature of 6°C is sufficient. Furthermore ROMIJN determined the viscosity of the streaming protoplasm by the type of plasmolysis. For WEBER's investigations led to the conclusion that in objects with low viscosity of the protoplasm the shape of the plasmolysed protoplast is round, whereas in those with high viscosity it is angular and concave, the protoplast clinging

as it were convulsively to the wall in different places, hence WEBER's term of cramp-plasmolysis. In this way it was established that *Nitella* had its maximum viscosity at 20°C, that is the temperature at which sensivity to a sudden change in temperature is greatest. Thus the view held by HILLE RIS LAMBERS, who ascribed the change in the velocity of streaming exclusively to changes in viscosity, can hardly be correct. It is true that below 10°C velocity decreases more strongly than above that temperature, and ROMIJN associated this inflection point in the graph with investigations of L. ARISZ [12] and H. G. DE JONG [303], regarding it as a result of gelation of the protoplast.

H. P. BOTTELIER [65] adopted an entirely different point of view in his work concerning the protoplasmic streaming in the epidermal cells of *Avena-* coleoptiles, which he studied in the laboratory of V. J. KONINGSBERGER [343]. When BOTTELIER examined this streaming in adult cells, he found that up to approximately 30°C there was a logarithmic rise with the temperature. The Q_{10} is 1.80, hence the process follows the rule of VAN 'T HOFF. [263] In studying these values in non adult cells, BOTTELIER found this curve applied only below a certain temperature level, and the younger the cells, the lower this temperature level. Above this level a rectilinear course is observed in young cells, the accelerating action of temperature being only very slight, hence it would appear as if some limiting factor had developed. From this BOTTELIER concluded that in adult cells a chemical process determines the rate of streaming, whilst with the rectilinear rise in young cells a process of diffusion is the limiting factor. He succeeded in demonstrating that this consists in the diffusion of oxygen towards the interior of the cell. Adult cells placed in an environment that is poor in oxygen behave as do young cells, while vice versa the curve of temperature of protoplasmic streaming in young cells takes a logarithmic course in an environment that is rich in oxygen. According to BOTTELIER the rectilinear connection between temperature and streaming, observed in the Characeae by other investigators may be due to the fact that successively different processes with a decreasing Q_{10} determine the the velocity of streaming.

Thus BOTTELIER demonstrated the close relationship between protoplasmic streaming and respiration and illustrated the fact that the flow comes to a standstill in the absence of oxygen, except in the case of those Characeae where the energy required is probably supplied by anaerobic respiration. However, although BOTTELIER's investigation is of great interest, it does not help much to explain the nature of the protoplasmic streaming nor that of the amoeboid movement of the naked protoplasts.

American workers, DU BUY [102] OLSON, [472] SWEENEY [639], THIMANN [649] and BONNER [59] assume that there are two respiratory systems, one of these or both supplying energy for streaming. OLSEN and DU BUY found that there is a parallelism in the effects of respiratory poisons

in the two systems. A solution of 0.01 N hydrocyanic acid applied to *Avena* coleoptiles decreases the streaming rate 50%, the respiratory 61% of the original. These authors conclude that the data show the presence of two respiratory systems one of which is governed by a non-cyanid sensitive enzyme and the other by a cyanid sensitive one. In their opinion the data demonstrate a direct dependence of protoplasmic streaming on the two respiratory systems. THIMANN on the contrary believes that only one of the respiratory systems is intimately associated with streaming.

It is impossible to mention all the theories of the physical mechanism of protoplasmic streaming; in the review of W. SEIFRIZ [597] 11 theories are presented and that of rhythmic contraction and relaxation is given preference.

On the ground of the investigation of VELTEN [671], HUGO DE VRIES had in the past century pointed to the importance of protoplasmic streaming for the transport of organic substances, as was noted in Chapter VI. He assumed that streaming of the protoplasm was a phenomenon of general occurrence in plant cells. This view was opposed by PFEFFER who argued that in the great majority of cases streaming, and especially rotation, did not set in until some time after the incision was made and streaming should, therefore, be regarded as a typical wound reaction. Later, many investigators supported PFEFFER's argument. This matter will be reverted to again in due course though not before another view has been mentioned, which in a sense may be regarded as exactly opposite, namely the view that protoplasmic streaming is the result of the transport of organic substances. This was referred to in Chapter VI in connection with VAN DEN HONERT's experiment.

Mention must also be made of the assumption of ALI C. A. KOK [340] that protoplasmic streaming does not accelerate the transport of caffeine or of lithium salts in the leaves of *Vallisneria spiralis*.

The problem as to whether protoplasmic streaming is peculiar to unstimulated or to stimulated cells was subjected to further research by H. FITTING [178], who used *Vallisneria* in his experiments. He found that if previously no streaming occurred, this set in as result of minute quantities of leaf extract. However, as streaming was also induced by all kind of other substances, FITTING first had to find a method by which he could cause streaming to cease or prevent it from arising. For this distilled water was required which, although not necessarily, chemically pure, for it might contain carbon dioxide or oxygen in solution, had to be free from certain substances; hence redistilling in a quartz still was necessary. The leaves of *Vallisneria* could be touched only with glass instruments and cutting was impossible, for traces of the leaf extract produce streaming, as already stated. If however, the leaves were cut, rinsed and kept some days in the redistilled water 96 to 100% of the cells showed no rotation.

Observation of the experimental material had to take place in green

light, for although light generally causes rotation, green light does so least of all. Furthermore, cells had to be observed with the aid of an immersion lens, of which the metal parts were japanned, otherwise the metal would act as a stimulus and cause streaming. Calcium ions in particular have a stimulating effect, but this is different from that exerted by leaf extract, the calcium ions being poisonous.

After the various difficulties mentioned above had been overcome and FITTING was able to stop protoplasmic streaming, it was found that the leaf extract was still active even when diluted to 1 in two million. Thus it was possible to specify more precisely the substance or substances present in the leaf extract, which caused this effect. It was found that these substances were stable when boiled and were non-volatile, though they could be destroyed by bacterial action. Moreover, they are not specific, as extract from *Elodea* acts on *Vallisneria* and vice versa. A further characteristic is that when, prepared with water, extracts from all kinds of objects, for instance purified paper, still have the same action.

FITTING then proceeded to examine an enormous number of organic substances of most diverse nature in order to ascertain which remained active even when very greatly diluted. By far the greatest effect was obtained with a number of α amino acids such as aspartic acid, or their amides, for instance glutamine, leucine, valine, though histidine excelled all of these. This substance is still active when diluted to 1 in 125 times 10^6, whereas glutamine retains its activity when diluted to 1 in 10^5.

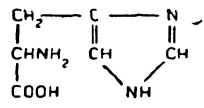


FIG. XXIX. Histidine

Tyrosine has hardly any effect, while l-aspartic acid is 200 times stronger than d-aspartic acid. Hence it is a specific sensitivity, a typical process of stimulation, which occurs. Sensitivity to a substance may become less specific by usage, so that after a certain time it ceases to have effect and a much stronger concentration is required to cause a fresh stimulus. It might be possible to speak of tonus in this connection, As cell-material made insensitive to the action of one substance is not insensitive to that of another, it is possible to ascertain what precisely is the active agent in the leaf extracts. Their action is so strong that, in view of the concentration of the substances present in them and the results of the above mentioned method, aspartic acid and histidine alone had to be taken into consideration. Of these two histidine is the more likely, or else there must be a third, still unknown substance with even stronger action than that of histidine. For FITTING found that the action of $\frac{1}{2 \times 10^4}$ leaf extract equals approximately 1 part of histidine in 10^6 parts of water. According to this calculation leaf extract should contain 2% of histidine.

Whatever this active principle may be, it has so far not been chemically identified in the extract.

As previously stated, these substances are present in such minute concentrations that they behave like true hormones. For example, in animal organisms thyroxine, the para-di-iodophenylester of di-iodo-tyrosine, is active in a dilution of 100 times 10^6, and in *Vallisneria* histidine (at least, if this is the substance concerned) is active in a concentration of 1 in 125 times 10^6. FITTING termed the phenomenon itself chemodinesis.

It is interesting to mention the results of BONNER's work in this matter. THIMANN's opinion is that when streaming ceases in leaves kept under water without stirring, the "bulk respiration" consumes oxygen so rapidly that none is available for "the special streaming respiration". Hence any agent which will reduce total respiration will allow oxygen to become available for the streaming system. Histidine was shown by BONNER to have this effect; it reduces total respiration in oat coleoptiles.

Curiously enough, *Elodea canadensis* was found by G. SCHWABE [593] to be stimulated by amino acids, particularly alanine, phenyl-alanine and tyrosine, but not by histidine. The substance concerned in this instance must, therefore, be akin, though not identical to those studied by FITTING in *Vallisneria*. In both plants leaf extracts have reciprocal action, so they must contain the two substances.

Finally it should be noted that the velocity of protoplasmic streaming should really be regarded as an average velocity. L. G. M. BAAS BECKING [35] found greatly varying velocities for different protoplasmic particles.

The apparent difficulty of reconciling the existence of a streaming protoplasm with the assumption of a fixed structure as the essential basis of vital phenomena, can be discussed only very briefly. A solution of this problem may possibly be found in the more recent views concerning protein chemistry and the structure of the protoplasm, explained in Chapter V in connection with the observations and considerations by FREY WYSSLING [186].

A great deal of controversy has arisen from the view that the structures in the cell-wall, which were studied by G. VAN ITERSON [288] during the past decades may have some connection with protoplasmic streaming. However, a discussion of this subject would lead us to far into the realm of anatomy. Suffice it to state that one of the recent investigations into the question is that by A. J. P. OORT [474] and P. A. ROELOFSEN [548]

(C) Movements of Nuclei and Chloroplasts.

Not only the protoplasm in the cell, but also various organs are capable of active, independent motion. In the previous century E. TANGL [444] studied this phenomenon in nuclei and found a reaction to wound-stimuli, called traumatotaxis. The classical example is the movement made by the nuclei of the epidermal cells of the bulbscales

of the onion, *Allium Cepa*, when these are pierced with a red-hot needle. Later, in 1911, G. RITTER [545] observed the chemotactic movements of the nuclei of different plants and G. HABERLANDT [222] pointed to the connection of this movement and the formation of secondary thickened layers in the wall nearest the nucleus.

More study has been devoted to the movements of chloroplasts. In the previous century E. STAHL [620] and A. F. W. SCHIMPER [575] were the principal workers in this province. According to them, the position of the one plate-shaped chloroplast present in *Mesocarpus* one of the Desmidiaceae, as well as the arrangement of the chloroplasts in Bryophyta such as *Funaria*, or in higher plants such as *Lemna* was principally determined by the direction and intensity of light, hence it was true phototaxis.

During the present century the subject was studied in particular by G. SENN [600] who concluded that at least part of these movements must be regarding as being chemotactic, and in agreement with the movements of nuclei, previously referred to, as pertaining to traumatotaxis, i.e. chemotaxis.

The chemotactic movements of the chloroplasts of *Funaria* had long been known and it was assumed that carbon dioxide, sulphates, malic acid, asparagine and monose exerted attraction. With Bryophyta, however, the position is a simple one compared to that in higher plants, the leaf of mosses having a thickness of only two layers of cells and being without epidermis.

SENN's conception is that in the higher plants the night position, or so-called apostrophe, in which the chloroplasts accumulate along the inner cell-walls, is the result of the attraction exerted on these plastides by cells in which assimilates are accumulated. Epidermal cells without chloroplasts are poorer in such assimilates and therefore have a repelling action. According to SENN the term chemotaxis would be rather more correct in this case.

With intensive illumination, however, the arrangement of the chloroplasts along those walls which are parallel to the direction of the light rays, the so-called parastrophe, would be mainly a phototactic phenomenon, as would also be the arrangement found in weak light, when the chloroplasts are ranged along those walls that are at right angles to the direction of the rays, the so-called diastrophe. The way in which these movements of nuclei and chloroplasts are brought about is totally unknown. In SENN's opinion the movement is an active one.

(D) Locomotor Movement by Means of Cilia or Flagella.

Cilia and flagella are appendages formed from the outer layer of the protoplast. They possess a gel structure and generally show double refraction. If there is a large number of such appendages, they are usually termed cilia, but if there is only one or a pair of larger ones, they are called flagella. Sometimes the cilia are grouped in bundles.

Both the double refraction and the structure of these appendages were studied particularly by FREY-WYSSLING [186]. From their X-ray spectra it appears that the cilia and flagella as well as the cell-walls are formed by crystalline particles.

The movement of cells possessing cilia and flagella is never rectilinear, but is in the form of a spiral around a longitudinal axis, and if the movement appears to be rectilinear, it is in reality a very steep spiral. This movement continues until an obstacle is encountered, when the organism either remains where it is or goes backwards with the hindmost pole in front, until the normal position is resumed. If an organism loses its flagellum or its cilia, the movement ceases. A flagellum which has been torn off will keep moving for a very short time, hence the flagellum may be regarded as the moving agent, though it needs the protoplast if the movement is to continue.

In the previous century the movement was studied by STRASBURGER [636] and later by W. PFEFFER, and in the present century it was subjected to exhaustive research particularly by P. METZNER [422]. This investigator imitated the movement by means of models and made observations in an innocuous though highly viscous environment which caused the movement to be slowed down, so that it became more readily discernible. METZNER also made use of a paraboloid condensor with a dark field, which enabled him to observe the space in which the flagellum moved. This was often found to have a conical surface. More recent still is the use of a stroboscope, with which the successive stages of the motion are rendered visible by means of flash light. By altering the frequency of the flashes the frequency of the motion may be observed. If the two frequencies are the same or if one is a multiple of the other, the same stage will be seen each time, so that it appears as if the flagellum is stationary. The experimental material studied most is *Spirillum volutans*, which has two sets of conjugated flagella, though other species have also been examined.

To explain the motion there are two theories which are directly opposed to each other. VL. ULEHLA [663] believes that the spiral shape of the flagellum or cilia is not permanent, but that successive contractions pass along its surface, the Flagellatae moving not like a screw but like a boat that is being sculled with one oar at the stern. On the other hand METZNER refers to the instance of *Spirillum volutans* where the flagella contract spirally one after another, the flagellum revolving 40 times and the body 13 times per second round its axis in opposite directions. Consequently, by virtue of the spiral shape of *Spirillum*, this would be rotated through the water.

METZNER assumes that in organisms with but a single flagellum contraction waves are propagated along it. The most favourable position is that in which the longitudinal axis of the flagellum forms an angle of $22\frac{1}{2}°$ with the direction of the motion, though this angle is often greater, as for example in *Spirillum volutans*, where it is about 40°.

With the Flagellatae, where the flagellum leads, the course of events must resemble that of a propellor steamer moving astern and the contraction waves must run in the opposite direction. However, with the Peridineae, Flagellatae with two flagella, the longitudinal flagellum which provides locomotion, does not make a spiral but a wave movement similar to the motion of the ceel, whilst the transverse flagellum causes rotation.

Although these observations appear to enhance our insight into the mechanism of the locomotion of Flagellatae and bacteria, the way in which the contraction of the flagellum itself arises is still entirely unknown. There is the possibility of rhythmic changes in tumescence, but this is purely hypothetical. The existence of any contractile substance such as that in muscle fibre is certainly out of question.

As regards the external conditions required for these movements, in the first place there must be a sufficient amount of water, although it is possible for the motion to continue if the cell itself is plasmolysed. This phenomenon may be due to the fact that the colloid gel of the flagellum or the cilia binds the water to a very great extent. A. FISCHER[174] observed that rigor occurred in a solution of 5–10% potassium nitrate. He ascribed this rigor to desiccation, but at first sight there is no difference between this and the rigor caused by narcosis.

The influence of oxygen on the motion is important. The movement of obligate anaerobionts is brought to a standstill even by a low tension of oxygen, whereas oxygen is essential to the motion of aerobionts. Those that are facultative aerobionts react to oxygen in various ways, some continuing to move for a while even when the available oxygen has been consumed, others being capable of growth without oxygen, but not of movement. The better the source of nutriment, the longer the motion will continue under those circumstances, hence it may be assumed that in such cases the necessary energy is obtained by anaerobic respiration. Sometimes it seems as if organisms are capable of binding oxygen, as are some bacteria containing pigments.

PFEFFER repeatedly studied these locomotor movements and in his textbook of plant physiology he made a distinction between a topic and a phobic movement. With the former, the organism has a certain capacity for orientation, but with the latter it can only recoil if a certain drop in concentration is exceeded.

With topic phototaxis the specimen has one particular spot, the eyespot, which is specially sensitive to the light-stimulus, causing the organism to move in a certain direction, either towards or away from the source of light. Possibly the fact that the shadow cone of the eyespot falls in a certain direction may have some bearing on this phenomenon. With chemical stimuli a topic movement is possible owing to the fact that the front and the back of the specimen are situated in different zones of concentration of the active substances; this is referred to as topic chemotaxis. In any case the specimen turns in a direction determ-

ined by the chemical stimulus and keeps on following that direction.

With phobic movements, on the other hand, there is no question of any definite capacity for orientation. The organism moves in some direction or other and continues to do so up to a threshold value characteristic of the specimen concerned, whether this be a chemical one, i.e. a certain concentration of the stimulating substances, or a photic one, when a certain intensity of light has been exceeded. Then the specimen goes backwards in a slightly different direction, which may or may not be due to pure chance, and continues to do so until once more a certain threshold value has been reached. Hence it may be said that an organism with a phobic reaction is confined between the limits of a certain zone of concentration. For instance, S. KUSANO [356] found that the sulphur bacterium *Thiospirillum* recoiled at a decrease in light-intensity of 1000–2000 meter-candles and also at one of 20–10 meter-candles. On the other hand, an organism with topic phototaxis will continue to move in the direction of the source of light and even swim right through the focus of the lens, as was observed by J. BUDER [88].

Presently it will be seen that the distinction between topic and phobic movements is not so sharp as PFEFFER believed it to be. A curious result was obtained by METZNER [422] who found that a colourless bacterium which did not react to light, showed phototaxis after treatment with eosin.

According to whether organisms move or do not move in light it is possible to distinguish between:

(1) negatively photo-kinetic organisms like gametes which become quiescent in light and start moving when it becomes dark again. Example *Haematococcus* sp;
(2) positively photo-kinetic organisms, which sooner or later become quiescent in the dark, like those of *Eudorina elegans*;
(3) photo-kinetically indifferent organisms of which the gametes begin to move under illumination, for instance after 3 minutes of artificial sunlight, as was demonstrated by MOEWUS [434], and also become mobile in the dark if oxygen and a special sugar are supplied. This will be discussed later on.

During the previous century PFEFFER had studied the movements of the spermatozoids of the ferns and had wondered why these are attracted to the neck of the archegonium. He tried to solve this question by filling fine capillaries with solutions of all kinds of substances in order to see to which capillaries the spermatozoids would move when they were swimming freely in an environment without those substances. He found that in half a minute an many as 60 spermatozoids had moved into a capillary containing 0.01–0.5% potassium malate, whilst other substances failed to attract this number. According to PFEFFER the lowest threshold value lay at 0.01%. He considered that the results of his investigation showed that the well-known law of WEBER-FECHNER pronounced by animal physiologists also held good in this instance and that there must be a certain ratio between the concentrations inside

and outside for chemical attraction to arise. For instance, there was attraction if the outside concentration was 0.0005% and inside 0.015%, and again if these were 0.005% and 0.15% respectively. However, later investigators did not agree with PFEFFER's results, for he had not taken into account the diffusion phenomena which arise at the opening of the capillary, causing the difference in concentration, to which spermatozoids react, to be much smaller. Moreover, PFEFFER believed that this was a case of purely topic chemotaxis (topochemotaxis or strophic chemotaxis), but the more recent investigations by W. D. HOYT [280] show that they are rather phobic movements (phobochemotaxis or apobatic chemotaxis)

According to PFEFFER a salt of malic acid was also the most active substance with other vascular Cryptogams such as *Salvinia* and *Isoetes*, but with Bryophyta he found that attraction was strongest with a solution of sucrose. A later investigation by B. LIDFORSS [375] showed that with *Equisetum* sp., the most active substance is likewise a salt of malic acid, whilst proteins have the strongest attraction with *Marchantia polymorpha*. BRUCHMANN [84] observed that citric acid was the active substance with *Lycopodium* sp.

Because the di-ethyl-ester of malic acid, which is not dissociated in an aqueous solution, does not cause attraction, PFEFFER concluded that the effect of salts of malic acid, must be attributed to the malate ion and not to the undissociated acid.

At the beginning of the present century the study of this problem of attraction of spermatozoids was resumed by R. BULLER [91], later by K. SHIBATA [603]. Both found that other substances also had this chemotactic action on the spermatozoids of ferns. Hence SHIBATA assumed that there were three different kinds of chemotactic sensitivity: one to the hydroxyl ions, one to the anions of malic acid and allied dicarboxylic acids, and finally one to the cations of potassium, rubidium, calcium and strontium, as well as to the alkaloids. By applying the method referred to above SHIBATA demonstrated that these different kinds of sensitivity were independent of each other.

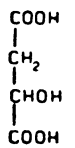


FIG. XXX. Malic acid

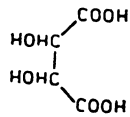


FIG. XXXI. Mesotartaric acid

Particularly interesting is the sensitivity of fern spermatozoids to malic acid and its allied acids, viz. fumaric acid and maleic acid, also mesotartaric acid, citraconic acid and mesaconic acid.

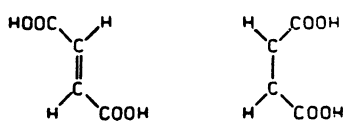


FIG. XXXII. Fumaric acid (trans position and Maleic acid (cis position)

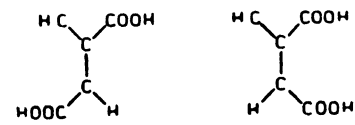


FIG. XXXIII. Mesaconic acid (trans position, Citraconic acid (cis position)

It is curious that although the spermatozoids of *Equisetum* react to the ions of malic and mesotartaric acid, they do not react to those of fumaric or maleic acid hence they do not react to acids with double bonds. On the contrary, *Isoetes* reacts to the ions of this type of organic acids in the trans-position, i.e. to fumaric and mesaconic acid. This clearly shows how totally different sensitivity may be to substances which merely differ in stereo-structure, which naturally calls to mind the old comparison of key and lock in connection with enzyme activity.

In regard to the greater sensitivity to different groups of organic substances, it should be noted that with *Marchantia*, besides its sentivity to proteins, already observed by LIDFORS [375], a sensitivity to the ions of potassium, rubidium and caesium was found by A. AKERMAN [2]. The last-named sensitivity was likewise encountered in the spermatozoids of ferns by SHIBATA. According to the latter, the spermatozoids of *Salvinia* are sensitive to calcium and strontium-ions, whilst those of *Equisetum* react to hydrogen-ions, those of *Isoetes* to hydroxyl-ions. Hence the matter is far more complicated than PFEFFER believed it to be.

In Chapter IV reference was made to the experiments by ENGELMANN[157] with bacteria which are specially sensitive to oxygen. These bacteria reach spots with an optimum tension of oxygen by means of chemotaxis. How sensitively they react to this element was shown by ENGELMANN's calculation, according to which there are instances where a reaction occurs to 10^{-18} mg. oxygen, i.e. approximately 300 molecules.

In regard to the different kinds of sensitivity to various substances, reference must be made to an investigation by H. KNIEP [333] concerning the properties of the so-called bacterium Z in this respect. This bacterium reacts positively to peptone, asparagine, ammonium-ions and calcium-ions as well as to phosphates, though it does not react to urea or glucose. Asparagine does not render it intensitive to phosphates or ammonium-ions, but ammonium chloride does cause insensitivity to ammonium nitrate. Hence there most probably are three kinds of sensitivity: one to phosphates, one to ammonium-ions or hydroxyl-ions and one to asparagine.

Another method of ascertaining whether one and the same kind of sensitivity is concerned is the use of two concentrations of different substances, each below the threshold value, to see if these two are additive. Determination of the threshold value, however, frequently gives rise to difficulties, as is shown by the controversies between the various investigators. In this respect the width of the capillaries used is of great importance.

In this connection C. SPRUIT [619] made some very instructive experiments with *Chlamydomonas variabilis* in the laboratory of F. A. F. C. WENT. He put 0.1 normal acid in the capillaries, whereby a spherical accumulation of gametes was observed at the open end. Similar capillaries with 0.1 normal acid were placed in distilled water which had

been coloured orange by means of a solution of methyl-red. As a result of the diffusion of the acid out of the capillary a red ring arose at approximately the same place where the *Chlamydomonas* accumulated in the first experiment. Therefore, this place is the transition point of methyl-red, which lies at a p_H of 5, from which it follows that the optimum concentration of acids, for *Chlamydomonas* lies at the same p_H.

In 1919 SPRUIT tried to prove that in the chemotaxis of this organism colloidal-chemical processes play an important part, perhaps even the major part. For it was found that the concentration of acids, influencing this chemotaxis, is extremely small, being 0.0001 to 0.00001 normal, and this is also found in protein colloids.

Cultures of *Chlamydomonas variabilis* have a positive geotaxis. In 10 minutes the gametes move actively towards the bottom of a test-tube placed in the dark. They also have a negative phototaxis and in an illuminated space they move to the dark side of the liquid culture. However, all kinds of circumstances such as the presence of diluted acid or alkali may prevent this geotaxis or phototaxis from arising, and this again points to processes of a colloidal-chemical nature.

In addition, *Chlamydomonas variabilis* has the property of adhering more or less to the glass wall of the test-tube, and this gelation, which is the chemical term for this property,is enhanced by a high degree of acidity. By taking advantage of these properties it is possible to obtain fairly pure cultures in a simple way. The test-tube containing the culture is placed first in a space with one-sided illumination, which causes the organisms to adhere to the dark side. Next, the liquid is poured off and distilled water is added whilst the tube is placed in the dark. The adherence then lessens and owing to their positive geotaxis the Flagellatae move towards the bottom of the tube, enabling the liquid with its impurities to be poured off.

SPRUIT placed the cultures in solutions where the influence of salts was combined with that of acid or alkali and it was possible to study their action on the geotaxis of the Flagellatae. The results obtained by him indicated that there was an additive influence of alkali and salt in the alkalinic region, in other words, one enhances sensitivity for the other. In the acid region the reverse occurred, acid and salt opposing each other's influence. This agrees with the combined action of salts and acid or alkali on globulins, observed by W. B. HARDY [236]. These are true lyophobic colloids, whilst the behaviour of the protoplasm for the rest shows much more similarity to that of lyophilic colloids, as explained above.

However, the problem as to whether in this case emulsoids (lyophilic colloids) of suspensoids (lyophobic colloids) are concerned, may be solved by ascertaining if the so called HOFMEISTER [264] series applies. In other words whether the anions influence these colloids in a way related to their lyotropism, viz. the ability to change the surface tension

of the water and to unite the water molecules into complexes.

For anions in an acid environment the HOFMEISTER series is as follows: SO_4'', acetate, Cl', ClO_3', NO_3', Br', I', CNS', the first of the series causing least and the last causing most swelling. In alkaline environment the series is reversed.

According to SPRUIT, the results obtained by him were in indication that lyophilic colloids are *not* involved in this instance, but it is questionable whether this conclusion is justified. FRANSJE VERSCHAFFELT [676], who some 10 years later made the same kind of experiments with *Chlamydomonas variabilis* in the present writer's laboratory, obtained results which do point to lyophilic colloids.

Finally the curious results of F. MOEWUS's [434] work should be mentioned. This investigator also experimented with various species of the genus *Chlamydomonas*, in particular with varieties of *Chlamydomonas eugametos*. These are Flagellatae with two flagella which serve for locomotion, but when cultivated on agar these flagella are absent and the organism is incapable of motion.

If the specimens cultivated on agar are transferred to a nutrient solution in the light, the flagella soon develop and with prolonged illumination copulation occurs, as a result of the presence of male and female specimens. Without illumination, however, they remain incapable of motion even in nutrient solutions.

MOEWUS wondered what it was that caused the development of the flagellum. He found that even in darkness this development is possible, provided oxygen is supplied and special sugars are added to the solution. With *Chlamydomonas eugametos* development of the flagella occurs best if gentiobiose is added, and to a lesser extent also upon addition of glucose, cellobiose and cellotriose, maltose, lactose, sucrose and raffinose.

However, with other species of the genus *Chlamydomonas* cellobiose is most effective. Addition of other sugars, for instance galactose, mannose or fructose, yielded no result. In the dark MOEWUS also achieved mobility by filtering a culture of Flagellatae which had become mobile in the light and adding the filtrate to cultures kept in darkness. Under those circumstances *Chlamydomonas eugametos* becomes mobile after only 4 to 5 minutes, though with other species it takes a little longer. Hence a substance which causes mobility must have been excreted in the culture subjected to illumination. What is this substance? By evaporation of 300 litres of the filtrate an orange coloured solution was obtained which upon hydrolysis was decomposed into a sugar and a pigment with the properties of the carotenoids. The spectrum indicated the presence of trans-crocetin, $C_{20}H_{24}O_4$.

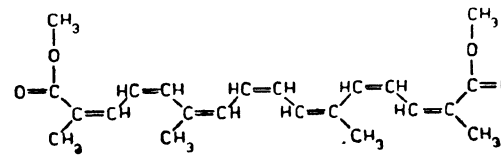


FIG. XXXIV. Crocetin

It seemed most likely that the substance concerned was the carotenoidglucoside crocin, which may be prepared from the stigmata of *Crocus sativus*, and this likelihood became a certainty when it was found that a 0.0001% solution of crocin was capable of causing the immobile gametes of *Chlamydomonas eugametos* to become mobile in darkness and in the presence of oxygen. The concentration required is very slight; since crocin is still active in a dilution of 1 in 250 trillions, it is possible to calculate that approximately 1 molecule per cell is effective, i.e. an even far smaller quantity than that required in ENGELMANN's[158] experiments, referred to previously. Hence there is every reason to speak of hormonal activity and the conclusions drawn at the end of Chapter VIII apply in this instance with even greater force.

Another problem was what rendered the gametes of *Chlamydomonas eugametos*, which had become mobile, capable of copulation. This requires longer illumination with blue or violet light, but what is the actual course of events?

MOEWUS naturally studied the action of a filtrate of those cells which had become capable of copulation as a result of illumination. He found that a filtrate of illuminated female cells rendered other female cells, which were kept in the dark, capable of copulation and that likewise a filtrate of illuminated male cells could induce other male cells to copulate in the dark. Moreover, after 4 minutes' illumination with blue or violet light a so-called female filtrate becomes inactive, but if illumination is continued, it turns into a male filtrate after 48 minutes and is totally inactive after 54 minutes. Hence it may be be said that illumination first causes a special female sexual substance to arise, which with prolonged illumination changes into a special male sexual substance, which in turn is destroyed by still further illumination. If filtration of a culture cultivated in darkness is carried out in red light, resulting in a filtrate which does not yet possess any copulatory action, it is possible to change this filtrate into a female filtrate by subjecting it to illumination with blue light for about 25 minutes and with further illumination this turns again into a male filtrate after 48 minutes. Hence these conversions may be effected in vitro.

The solution of this problem was a follows. With the variety called *Chlamydomonas eugametos simplex* 3 parts of the inactive preliminary stage plus 1 part of the inactive final stage form a female filtrate, 1 part of the inactive preliminary stage plus 3 parts of the inactive final stage forming a male filtrate.

According to its absorption spectrum the inactive substance of the final stage is the trans-crocetin dimethylester, and this was confirmed by experiments in which the pure substance was mixed with the respective quantities of the preliminary stage. In view of investigations by R. KÜHN [353] and A. WINTERSTEIN [736] concerning the conversion of the dimethyl-ester of cis-crocetin into that of trans-crocetin by means

of blue or violet light, MOEWUS assumed that the inactive substance of the preliminary stage was this cis-crocetin dimethyl-ester. This assumption was also confirmed experimentally. The dilution in which these sexual substances are active is 1 in approximately 33,000 millions, hence by far not as diluted as that of crocin referred to in connection with the induction of mobility in gametes.

It would lead us too far to include the other species of the genus *Chlamydomonas* in this discussion, the more so as this would lead us into the very extensive realm of genetics, a subject which is dealt with in the chapter on propagation in HUGO DE VRIES's Textbook. However, the 3rd edition of this Textbook appeared 5 years before genetical science began to develop as a result of the re-discovery of MENDEL's law, in which HUGO DE VRIES played an important part. As it is, genetics lie outside the scope of this book.

However, a few points concerning the sexual substances of this genus *Chlamydomonas* will have to be considered. Firstly, as to what is the function of this formation of a cis-trans mixture of crocetin dimethyl-ester. There is every reason in this instance to assume chemotaxis, the exertion of attraction by these substances, such as also occurs in the formation of the special substance in the neck of the archegonium of the vascular Cryptogams. When MOEWUS made experiments in this direction, he observed that the sugars capable of rendering immobile cells mobile in the dark, provided oxygen is present, may also serve as means of attraction. With these Flagellatae gentiobiose exerts attraction in a concentration of 0.0001 molar, and crocin does the same, although it is necessary for the solution in the capillary to be approximately 10.000 times stronger than that in the environment to induce attraction. Hence MOEWUS concluded that copulation occurs only if chemotaxis is positive. In other words, the cis-trans mixture of crocetin dimethyl-ester has the effect of causing mutual attraction of these gametes.

From this it might be concluded that these mixtures determine the sex of the gametes, but this is not so. They do no more than to prepare the female and the male cells for copulation, in the concentrations mentioned. However, according to MOEWUS, there are in addition, special substances which determine the sex, the so-called termones, one the one hand an androtermone, on the other hand a gyno-termone.

Our discussion would become too detailed if all this had to be explained in respect of the different monoecious and dioecious species and varieties of the genus *Chlamydomonas*. But we can draw the conclusion that if an agar culture of a dioecious species is placed in an illuminated solution, protocrocin is decomposed on the one hand into crocin, the substance which induces mobility, on the other hand into a termone. In the presence of the

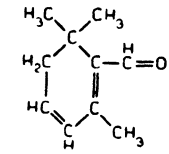


FIG. XXXV. Safranal

female gene, this will be the gynotermone, a substance which seems akin to picrocrocin, the glucoside of safranal.

On the contrary, if the male gene is present, the androtermone oxysafranal is formed by decomposition of the gynotermone. These substances, gynotermone and androtermone should not be confused with the gamones mentioned first, the cis- and trans-crocetin dimethyl-esters.

It is obvious that MOEWUS's work has done much to enhance our insight into the analysis of the fertilization phenomena of these Flagellatae. Moreover, it serves to illustrate the fact that in this connection a very great deal remains completely obscure. For what is the course of events in the innumerable other species?

Final Observations.

The subject terminating the discussion in the last section is a typical illustration of the well-known image of the circle, the circumference of which encloses all that is known, whilst all that lies outside represents the unknown. As our knowledge grows the circle expands but so does the circumference, that is the locus of all these points where it borders on the unknown. Mutatis mutandis, this applies to the whole plant physiology.

Summarising the progress of plant physiology during the past 50 years we are struck first of all by the enormous increase of our factual knowledge in every respect and next to this, by the greater penetration of our understanding and the classification of our insight in many branches of the subject. Reference to chapters on respiration and on growth will illustrate the complete change which our views have undergone.

As regards respiration, a thorough analysis of the entire chemical process has been effected, resulting in its reduction to a number of individual reactions, each of them realizable in vitro.

Our ideas concerning the nature of the process of growth have been quickened and greatly modified by the discovery of auxins and bios substances. The new hormone theory has led to a new understanding and a wholly different explanation of the phenomena that constitute growth and movement in plants, although it should be remembered that in plant physiology — as indeed in science generally — what we call an explanation really is a scheme of thought into which all the known facts can be fitted most satisfactorily. One thing, however, is still wholly mysterious to us: the interdependence, the causal connection of all these processes. How it is that the living protoplast organizes itself into a well-coordinated system which sets and keeps itself going? This is the idea of totality, so characteristic of modern biology which to-day well-nigh dominates plant physiology. In the chapter dealing with the metabolism of heterotrophic plants we have briefly set forth A. J. KLUIJVER's[331] attempt

to arrive at a satisfactory conception of the harmonious interaction of vital processes.

This of course raises important questions of principle which cannot be gone into here. Suffice it to say that the reader will find these questions discussed extensively in L. VON BERTALANFFY's [43] well-known work entitled "Theoretische Biologie". For the same reason we need not enter into a discussion of the vitalistic principle of "Entelechie", once more brought into prominence by H. DRIESCH [146] but pronounced unscientific by the mechanistic school of thought. v. BECKING's [35] Notes on the determined and the undetermined in Biology.

Be this as it may, the combined work of plant physiologists has often albeit by many devious ways, enabled us to reduce the facts furnished by nature and experiment to processes of the living protoplasm, and attempts are now being made to achieve a conception of its structure. Whether the human mind is also capable of conceiving an image of the complex of all these physiological processes in its entirety — who shall say?

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In this index the years of birth and death of each scientist are given, wherever these could be ascertained. In some cases only the former or the latter were to be found in the usual works of reference; this is indicated as follows (1870—) *or* (—1930). *Where neither data was known, the year given (in brackets) indicates the date of the most important publication by the scientist in question.*

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