EXPERIMENTAL BIOLOGY MONOGRAPHS

PHYTOHORMONES

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F. W. Went
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June, 1937

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CHAPTER I

INTRODUCTION

The field of plant hormones is perhaps now at the stage of its most rapid development. The number of facts is becoming so large, and their distribution through the literature so scattered, that there is a danger of losing sight of the general trend. We shall attempt not so much to give a detailed historical account as, rather, to present the field from the point of view of workers in it. Where matters of hypothesis are concerned, our personal views will necessarily be emphasized, but opposing views will be given an opportunity for the reader's consideration. In matters of fact, as also in regard to credit and priority, every attempt will be made to give as fair and accurate an account as possible, both of the experiments and of the concepts of the different workers. For the sake of completeness many new experiments have been included. These are designated as *u* (unpublished). Some idea of the amount of work which has been done in this field may be gained from the statement that the contents of this book are based on actual measurements of the responses of about one million plants. The growing interest in the field is exemplified by the fact that the bibliography includes references to 77 publications dated 1936. Since equally detailed and critical treatment cannot be accorded to all of this material, we have naturally laid emphasis on what appears to be the most important work.

A. Outline of the Book

Our review will deal only with the hormones of higher plants. We shall first trace the development of the leading idea that correlations in plants are due to the influence of special substances (Chapter II).[1] We shall try to show how experiments along four different and apparently unrelated lines,—correlation proper, organ formation, tropisms, and normal growth,—have gradually come together and been unified into a complete picture of hormone activity as we now know it. Next we shall consider the methods for the assay of these substances, treating them in sufficient detail for experimental use (Chapter III). Since most of these methods are founded upon cell elongation, and all other work has had its foundation thereon, it is natural to consider cell elongation in some detail first.

The best demonstration of the effectiveness of these assay methods has been the working out of the chemical nature of the active substances (Chapter VII). The success of the chemical attack has made it possible for all the experiments described subsequently to be checked by use of the pure compounds; this has had the effect of making the conclusions clear-cut and has avoided the difficulty of working with unknown extracts and mixtures.

Parallel and simultaneous with work on the above lines, the rôle of the active substances in various aspects of plant growth has been elucidated (Chapter V), beginning with their formation (Chapter IV) and movement in plant tissue (Chapter VI). The latter phenomenon is of special interest, firstly because it offers an example of a naturally occurring substance whose movement can be followed quantitatively throughout the plant, and secondly because of the causal relation between the polarity of this transport and the well-known polarity of plant structures. One of the most interesting aspects of the subject has been the attempt to analyze the various reactions intervening between the auxin and its final effect,—growth (Chapter VIII). In this, knowledge of the chemical nature of the substances has played an essential part. In close connection with problems of cell elonga-

[1] References such as VIII *G* or III *C* refer to chapter (Roman numbers) and section (letters).

tion and transport come the tropisms, particularly the reactions of plants to gravity and light, insofar as they are caused by unequal growth (Chapter X).

Further, these same active substances play an important rôle in a number of other correlations in plants, particularly in the formation of roots (Chapter XI), in bud inhibition (Chapter XII), and in the stimulation of cambial activity (Chapter XIII). There are also a number of other phenomena not so well understood.

Finally the findings and general conclusions will be compared with those from other fields, bringing the work on plant hormones into the realm of general physiology (Chapter XIV).

B. Definitions

The definition of *hormone* which we propose to apply is this: *a hormone is a substance which, being produced in any one part of the organism, is transferred to another part and there influences a specific physiological process.* This is essentially the definition of Bayliss and Starling (1904): "the peculiarity of these substances (hormones) is that they are produced in one organ and carried by the blood current to another organ, on which their effect is manifested" (Bayliss, 1927, p. 712). There is, of course, no blood stream in plants, but, as Bayliss emphasizes, "these hormones are characterized by the property of serving as chemical *messengers*, by which the activity of certain organs is coördinated with that of others."[1] There is no strict necessity for the production of the hormone in specialized organs, since even if all the cells of the plant should produce it, the phenomenon of polarity would bring about its specific distribution. In general, however, the points of production and response are spatially separated.

To avoid the possibility of confusion with animal mechanisms the term phytohormones has been introduced for

[1] The conception of hormones has recently been somewhat broadened (see Huxley, 1935).

such substances in plants. However, since in this book we shall deal only with the plant kingdom, the prefix can suitably be dropped. Thus our conception of hormone is essentially that of a correlation carrier, where correlation (as used in regard to plants) is defined as the influence exerted by one part of the plant upon another,—not in the sense of statistical correlation, but in the sense of causal relationship.

In the beginning of the work in this field, the noncommittal terms growth substance, Wuchsstoff, growth regulator, and growth hormone were used, but as our knowledge developed, it became clear that the substances causing cell elongation must be regarded as a separate group. Since recent work indicates that this group is heterogeneous, the term *auxins*, first suggested by Kögl and Haagen Smit (1931), will be arbitrarily restricted to those substances which bring about the specific growth reaction which is conveniently measurable by the curvature of *Avena* coleoptiles. Whenever used in the physiological sense, the terms growth substance (g.s.) and growth hormone will be used throughout this book in the sense of auxins. The term *Wuchsstoff* in particular has been used for some of the growth substances of lower plants, such as Bios, but it cannot be too strongly emphasized that only those substances whose activity is determined on higher plants, preferably by the standard methods which are described in Chapter III of this book, can be termed auxins.

C. Previous Reviews of the Field

The rapid development of the field has resulted, as would be expected, in the publication of a number of reviews and summaries. Such reviews have rarely more than temporary interest, and many of these are already only of historical value (Babička, 1934; Cholodny, 1935*a*; Kögl, 1932, 1932*a*, 1933, 1933*a*, 1933*b*, 1933*c*; Laibach, 1934; Loewe, 1933; Malowan, 1934; Pisek, 1929; Snow, 1932; Söding, 1927, 1932; F. A. F. C. Went, 1927, 1930, 1931, 1932, 1932*a*, 1932*b*, 1933, 1933*a*).

The newer and more extensive reviews are those of du Buy and Nuernbergk (1932, 1934, 1935), Erxleben (1935), von Guttenberg (1932, 1933, 1934, 1935, 1936), Heyn (1936), Jost (1935), Kögl (1935, 1935*a*, 1936), Haagen Smit (1935), Stiles (1935), Thimann (1935, 1936*b*), Went[1] (1935*b*, 1936*a*), and F. A. F. C. Went[1] (1934, 1935).

The most complete account which has appeared up to now is that of Boysen Jensen (1935, translated and extended by Avery and Burkholder, 1936). We do not feel, however, that Boysen Jensen's publication makes our book superfluous, for several reasons. In the first place, it does not attempt to do more than review the past work, while in this book our aim is rather to analyze and integrate the material. In the second place, its scope is restricted largely to the rôle of hormones in growth and tropisms, while, as was stated above, the field has recently developed in quite different directions, which necessitates a revision and broadening of our ideas. Lastly, it appears from book reviews (Söding, 1935*b*; Umrath, 1935) that one of the principal impressions which the book has made is in regard to the question of priority in the discovery of the auxins. We feel that the gradual unfolding of the current conceptions and the coöperation of different workers has made it impossible to credit any one person with such a discovery, and it is to be hoped that the reader of this book will gain the impression of a steady and collective advance rather than of individual contributions.

[1] References to Went (without initials) refer to F. W. Went, the papers of F. A. F. C. Went being cited with initials.

CHAPTER II

DEVELOPMENT OF THE HORMONE CONCEPT

A. Correlation and Formative Substances

The idea that the phenomenon of correlation is brought about by substances or "saps" is by no means new. No detailed consideration need be given to the very vague idea of Malpighi (1675) nor to the artistic conceptions of Agricola (1716) of a "*materia ad radices promovendas.*" Careful experiments, however, were carried out by Duhamel du Monceau (1758), whose sound scientific reasoning led him to conceive of correlation as brought about by two saps, one moving downward, the other upward. The former was elaborated in the leaves and, after passing downward through the cortex, was used for the nutrition of the roots. If, however, this downward stream were intercepted by ringing or other means, it caused the swellings, callus, and root formation which he observed above the point of interception (see

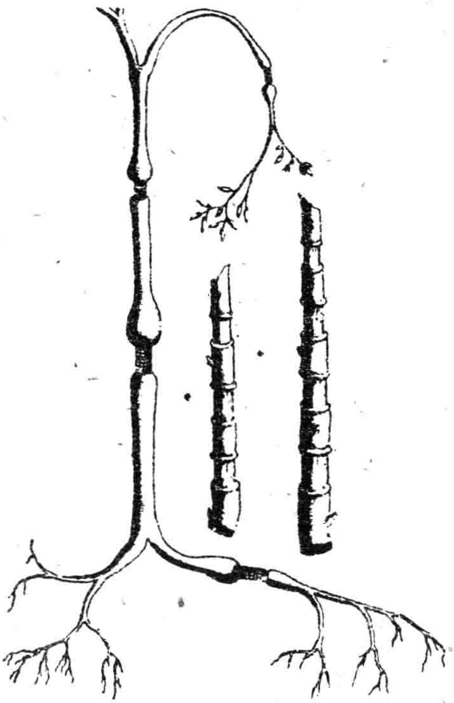

Fig. 1. The first published drawings of correlations in plants. Swellings occur above but not below ring wounds, and in isolated pieces of bark they are most marked when leaves or buds are present. (From Duhamel du Monceau, 1758.)

Figure 1). So much stress was laid on the root formation that the swellings and callus were considered as "being much of the nature of roots" (Bk. IV, Ch. V). During the next hundred years the physiological concept of correlation seems to have been lost, the emphasis being placed on morphology, *i.e.* upon the inherent nature of the tissues themselves. The discovery of sieve tubes by Hartig and von Mohl led them to the opinion that there was indeed a downward-moving sap, and this was later proven by Hanstein (1860). The content of this sap, however, was studied from the viewpoint of organic food materials rather than that of its correlating functions.

At this time began the period of rapid development in plant physiology. The phenomenon of correlation was studied in greater detail and Sachs (1880, 1882, 1893) brought forward a complete theory, a modern version of Duhamel's views, which covered most of the known facts of morphogenesis and correlation, and can still be regarded as a modern treatment of the subject. Sachs' great achievement was that he applied the laws of causality to morphology. His starting point was the thesis that "morphological differences between plant organs are due to corresponding differences in their material composition, which must be already present at the time of initiation, even though at this stage chemical reactions and other crude methods fail to show any differences." To account for these differences he assumed the existence of root-forming, flower-forming, and other substances, which move in different directions through the plant. For example, the former would be formed in leaves, and would move towards the base of stems. If a cut be made in a twig, this will be "an obstacle for further downward movement," and roots will be formed above the cut. Light and gravity were assumed to affect the distribution of these special substances. With only two assumptions: 1, the existence of organ-forming substances which, in minute amounts, direct development, and 2, polar distribution of these substances,—a distribution which may be modified by external forces such as light and gravity,—correlations, normal development, galls and monstrosities were brought into one picture.

In his very remarkable publications on galls Beijerinck (1888, 1897) elaborates the idea of "growth-enzymes."

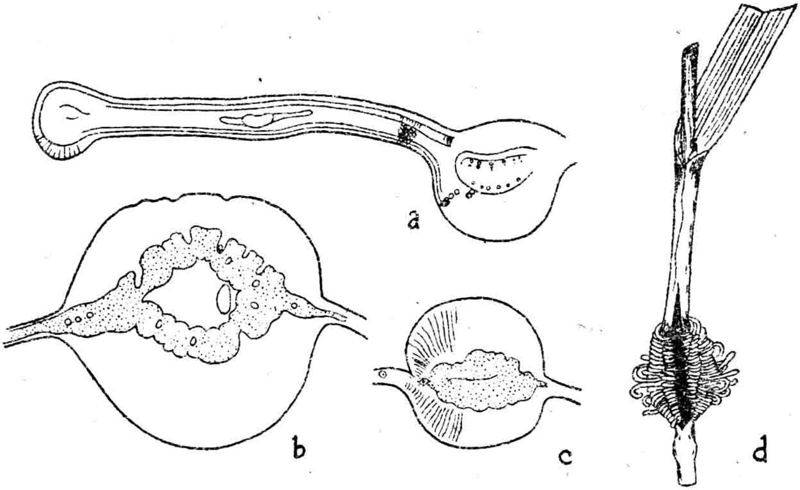

FIG. 2. a, b, c, gall of *Nematus capreae* on leaf of *Salix*. a, egg deposited with some mucilage in mesophyll of young leaf; b, mature gall before hatching of larva; c, gall in which by accident no egg has been deposited. The mucilage excreted by the gall-wasp has caused formation of an almost complete gall. This excretion is the first published example of an organ-forming substance. (From Beijerinck, 1888.) d, gall of *Cecidomyia Poae* on stem of *Poa*, showing excessive root formation. (From Beijerinck, 1885.)

While he originally thought (1886) that "it can not be doubted that nutritive stimuli must be considered as the primary cause" of root formation when parts of plants are cut off from the parent-plant, he afterwards modified his views in the direction of those of Sachs. In the case of the *Capreae*-gall on *Salix* (Beijerinck, 1888) he considers the development of the gall (see Figure 2) to be caused by "a protein, whose action differs from that of ordinary proteins, which only form an equivalent amount of protoplasm, and resembles that of an enzyme, whose effect is quantitatively of a different order of magnitude from the amount of active material." Thus we have to do here with a "material stimulus" (or as we should say now, a stimulating material) (1888, p. 132). Beijerinck designates this protein as a "growth enzyme." Later (1897) he extended this view to the development of organisms in general, and stated that "form is determined by liquid substances, which move freely through considerable numbers of cells in growing tissues" (1897, p. 203).

Except for these observations of Beijerinck no direct evidence for the existence of such special substances as Sachs had postulated was obtained for nearly 40 years. On the other hand, at about the same period, the existence of polarity in correlation phenomena was proven, both for whole organs, and for each separate cell of a transplant, by Vöchting (1878, 1884, 1892, 1908). His work, however, is primarily concerned with inherent morphological polarity, rather than with its physiological basis.

After the time of Sachs and Vöchting most of the studies on correlation laid emphasis on nutritional factors. Goebel (1908), for instance, in discussing quantitative correlations, says "of the numerous organ initials, many remain undeveloped because the building materials, which they need for their development, go to others which can 'attract' these materials more powerfully." Similar views, involving also the nitrogen content of the plant, *i.e.* "the carbon: nitrogen ratio," have been generally held by American workers (*cf. e.g.* Kraus and Kraybill, 1918). While their experiments show that there is a parallelism between a given carbon: nitrogen ratio and a given type of growth, no causal relation has been shown to exist.

B. Tropisms

About 1880 it began to be realized that tropisms were to be regarded as a special kind of correlation phenomenon. This aspect of tropisms was particularly emphasized by C. Darwin (1880). Both for roots and shoots he was able to show that the effects of light and gravity are perceived by the tip, and that the stimulus is transmitted to the lower regions, which then react. "We must, therefore, conclude that when seedlings are freely exposed to a lateral light, some influence is transmitted from the upper to the lower part, causing the latter to bend" (p. 474). In regard to geotropism of roots, he concludes "that it is the tip alone which is acted on, and that this part transmits some influence to the adjoining parts, causing them to curve downwards" (p. 545). At first Darwin's statements met with much opposition, but Rothert (1894), working with phototropism of shoots, confirmed completely the separation between the zones which perceive and those which react. The connection between these processes was envisaged by Fitting (1907) as being due to a polarity set up by the light stimulus, which "spread out" from cell to cell.

Fitting's work was closely followed by the experiments of Boysen Jensen (1910, 1911, 1913) which showed that a

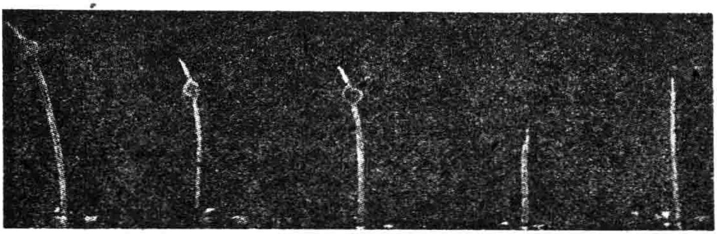

FIG. 3. First experimental demonstration of transmission of the phototropic stimulus across a wound gap. Five *Avena* coleoptiles were decapitated, and the tips replaced upon the three plants to the left, the wound being covered with cocoa-butter. Two plants to the right as controls. On illumination of the tips only, from the left, the plants with tips replaced show curvature in the base, the controls not. (From Boysen Jensen, *Ber. d. bot. Ges. 31*: 559–566, 1913; and *Growth Hormones in Plants*, tr. Avery and Burkholder, McGraw-Hill, 1936.)

phototropic stimulus can be transmitted across a wound gap. Boysen Jensen cut off the tips of *Avena* coleoptiles and stuck them on again with gelatin (see Figure 3). He then illuminated the tip only and showed that curvature appeared not only in the tip but also in the base. From this he concluded that "the transmission of the irritation is of a material nature produced by concentration changes in the coleop-