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The Relationship of Auximones and Phototropic Responses to Plant Growth

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THE RELATIONSHIP OF AUXIMONES AND PHOTOTROPIC RESPONSES TO PLANT GROWTH

This paper is submitted to the Faculty of Ursinus College in partial fulfillment of the requirements for honors in the Department of Biology

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Introduction

To select two definite topics of plant physiology and write of their combined effect on plant growth may superficially appear to be a simple and integrated task. This, however, did not prove to be the case in the preparation of this paper. My aim was to present, with all necessary ramifications the exact nature and function of auximones and phototropic responses in plant growth. I have purposely divided this paper into three phases; the first dealing with auximones; the second with phototropic responses; and the third consisting of a summary and the general conclusions obtained from experimentation.

I have in various parts of this paper interspersed drawings and tables compiled from my experimental works. In many places great portions of written material were replaced by tables of results obtained. This fact while greatly reducing the length of the paper is of great importance to the reader since the tables show in a clear concise form the experimental results.

I would like to advise the reader that I have merely scratched the surface of one aspect of plant physiology. There is more advanced work being done every day and almost before this paper is complete some of the findings may be termed obsolete. However, it is my wish that these results and conclusions may form a basis for some further work in the field of the relationship of auximones and phototropic responses to plant growth.

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List of Plants Used in Experimentation

Avena sativa --- Oat seeds

Raphanus sativus --- Radish seeds

Impatiens biflora --- Spotted jewel weed

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PART I ---- AUXIMONES

Within the past two decades, many experiments have been made which show that in addition to the foods and allied materials plants need still other materials in order to develop normally and completely. These materials seem in some cases to be taken into the body along with the food and in others to be manufactured inside the body. These materials not only have a helpful or beneficial effect upon the physiological processes of the plant which produces them, but also upon the animals which get these substances form the plants. Depending upon their function, their method of operation, and the way in which they are produced, these accessory factors have been variously treated by different workers under the names of hormones, vitamins, and auximones. Whether these are all distinct substances remains to be settled, but the general nature of the materials is the same. It is my purpose here to discuss solely the auximones.

Growth Hormones, Auxin, Its Action and Chemical Composition. --

The growth of cells in the zone of elongation depends most intimately upon the activity of the zone situated above it. If the top of the growing stem is cut, the elongation of the zone situated below it will be retarded and accelerated again only in case processes of regeneration occur on the cut end." Such an inhibition in growth can be clearly observed on peduncles after removal of the flower bud, and on the coleoptile of cereals protruding from the soil a few centimeters".[1.]

1. Maximov, Plant Physiology (New York,1938) Page123.

The coleoptile represents the first leaf of grass seedlings. It is colorless and possesses the shape of a hollow cylinder passing at the tip end into a complete cone. It is similar to a finger of a glove and serves for boring through the soil that covers the seed and protecting the tip of the first green leaf that grows up through its cavity. The growth of the coleoptile is mostly at its base. It is very rapid at the beginning, being greater than the growth of the forst leaf. Later on, its growth slows down; and then the first leaf overtakes it, ruptures the tip, and appears outside. After this the growth of the coleoptile ceases completely.

Boysen-Jensen (1910) observed the fact that growth of a decapitated coleoptile may be appreciably hastened if the cut tip is stuck back on the cut end by means of a drop of water or gelatin.

However, if a thin piece of tin foil or mica is placed between the cut end and the tip, acceleration will not be observed Hence, Boysen-Jensen drew the conclusion that the tip of the coleoptile excretes a special substance that accelerated growth and is capable of diffusing through a layer of water or gelatin.

Procedure:

Oat seedlings were sprouted in darkness at 40°C. without any nourishing medium. The seeds were placed on wet toweling and moistened from time to time. Within 48 hours sprouts appeared and were treated as follows:

The extreme tips of the coleoptile were cut off at a length of approximately 5-10 mm. These tips were then placed in a 10% gelatin solution and remounted on the coleoptile to one side of the central axis. These growing sprouts were observed and the following data recorded in tabular form:

Specimen number	Date	Growth in length	Curvature produced
1	4/14/39	1 cm.	4°
2		1.3	2°
3		.8	3°
4		.5	7°
5		.5	7°
6		1	4°
7		.7	3°
8		.8	3°
1	4/17/39	3 cm	8°
2		3.5	7°
3		2.7	5°
4		3.1	10°
5		3.1	10°
6		2.1	9°
7		4	4°
8		4.1	5°
1	4/19/39	7 cm	8°
2		2.5 cm.	7°
3		6.1	6°
4		5.7	10°
5		5.5	10°
6		6.2	9°
7		7.8	5°
8		5	5°

GROWTH ACCELERATION AND BENDING DUE TO AUXIN

(Oat seedlings and 10% gelatin solution used throughout)

Conditions:

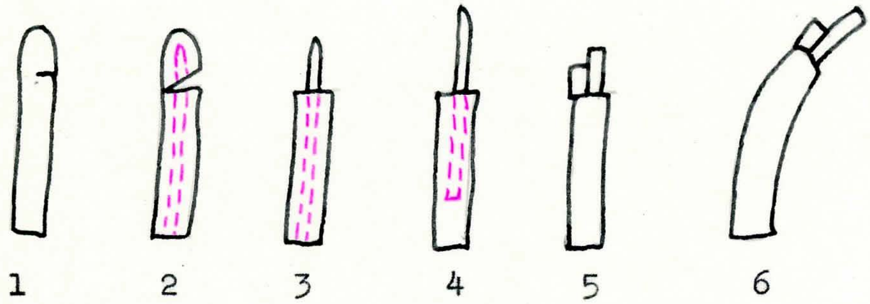
Temperature: 22° - 27° C.

Light: Normal sunlight

Nutrition: Planted in humus and watered periodically.

Bending of the oat coleoptile under the influence of auxin, 1, contained in an agar block, compared with agar alone 2.

Tip cut and remounted with gelatin.



1, coleoptile with incision; 2,3, decapitated coleoptiles; 3, first leaf partially pulled out; 4, first leaf partially pulled out; 5, agar cube placed on one side of the coleoptile; 6, result, bending of the coleoptile.

The explanation of growth-promoting substance, or growth hormone, has advanced especially rapidly during recent years, cheifly in connection with the work of the Dutch physiologist Went and his coworkers, and of Cholodny in Russia. To obtain the growth hormone in quantities sufficient for obvious display of its activity, Went applied the following procedure. The cut tips of oat and corn coleoptiles about 1 to 2 mm. in length were placed on a thin lamina of agar gel and remained thus for about 1 hour. After this the lamina was cut into separate small cubes, each of which contained a certain amount of the hormone. Such blocks, when placed eccentrically on decapitated seedlings, provoke a growth curvature (see prededing table). The amount of hormone sufficient to produce a curvature of 10 degrees in an oat coleoptile was defined as 1 "oat unit". Such blocks impregnated with the growth hormone were used to study some of its properties. It proved to be fairly stable, not destroyed by boiling. The rate of its diffusion through agar-gel was used to determine its molecular weight, which proved to be about 350.

Growth hormones may be formed not only in tops but also in several other parts of the plant organs. Thus according to the experiments of Cholodny, the substance is formed in definite elements of the phloem in hypocotyls of lupines and sunflowers. It is by means of a special borer the special central cylinder with all its elements is removed from a cut seedling, the growth rate is appreciably inhibited. Now if the cut tip of a corn coleoptile is placed in the center of such a hollow stem, growth of the stem will be considerably accelerated under the influence of the growth hormone excreted by this tip.

These experiments show that the growth hormone is not specific; the excretion of the tip of the corn coleoptile increases the growth rate of stems of the lupine and sunflower, and the head of the dandelion can accelerate the growth of the peduncle of the poppy or the coleoptile of oats. The growth-promoting substance is capable of moving only in the direction from the morphological top to the base, independent of the orientation of the organ in space. According to Went, the mechanism of its activity consists in the softening of the cell walls, which facilitates their expansion. This hormone is utilized in the process of growth and the lower -lying zones are provided with smaller quantities than those situated higher. This explains the distribution of growth in the elongating organ, the grand period of growth t hat is so often discussed in connection with plant growth.

It is interesting to note that the root tip excretes a substance that does not accelerate but inhibits growth of the root zone. This is dependent not upon the properties of the substance but on the peculiarities of the cells of the zone of elongation. Experimentally Cholodny displayed that cut tips of roots accelerate the growth of decapitated stem bases, and, conversely, that tips of stems inhibit the growth of decapitated roots. Thus the growth hormones proves to be identical, but various organs react differently to the influence of this substance.

The presence of the growth hormone may be revealed not only in the tops of plant organs but likewise in other parts of the plant and in secretions of the animal organism, e.g., in the saliva. But the discovery of this hormone in the urine of man and animals was of special significance, for it permitted the Dutch chemist Kogl to obtain it in quantities sufficient for detailed chemical study. In its preparation, he made use of its solubility in ether and insolubility in benzene, its capacity of giving insoluble salts with lead and soluble salts with calcium, etc. After a series of complicated manipulations, Kogl finally obtained 250 mg. of a completely pure crystalline hormone, which he designated "auxin", and established its chemical nature. It proved to be a monobasic acid of the empirical formula, $C_{18}H_{32}O_5$, easily transforming, when stored, into an isomeric completely inactive form. Urine contains on the average about 2 mg. of auxin per liter. Subsequently, several auxin-like substances have been identified of which indole-3-acetic acid is the one most widely used in experimental and practical work. It stimulated roots and causes callus and gall formation on stems of plants.

The physiological action of pure crystalline auxin is very intense. According to Kogl, 1 mg. of auxin contains about 50 million oat units, thus corresponding to an amount of hormone contained in 7 million coleoptiles of corn, for each such tip contains about 7 oat units.

Auxin gets into urine probably form the plants used for food. It is especially abundantly excreted when large amounts of vegetable oils are consumed. But it is probably that bacteria inhabiting the digestive tract also participate in its formation; for substances similar to auxin were found among the products of the vital activity of different microorganisms, especially of bacteria and molds.

The growth hormone is capable of moving through the plant only in one direction, from the proximal end toward the base and never moves in the reverse direction from the base to the top. According to Cholodny, this is connected with the distribution of the electric potentials in the stem, the top being charged negatively in relation to the base. That is why the anions of auxin, representing a weak acid, must move cataphoretically within the stem in the direction of the positive pole, i.e., to the base. This opinion is confirmed by the fact that a vertically standing coleoptile of oats, placed between elecrodes with a high potential difference between them, curve in the following manner: The side of the coleoptile turned to this pole charges negatively through an induced charge; and the flow of the hormone is directed to the opposite side, promoting an acceleration of its growth.

Auxin produces an accelerating effect only upon the stage of elongation and does not influence the division of cells, which determines the stage of meristematic growth. Accordaing to some authors (Haberlandt, Cholodny), plants possess specific hormones that excite cell division. These hormones are elaborated in the pholoem cells (leptome) of the conductive tissue and have therefore been designated "leptohormones". Their activity explains the fact that, in injured plant organs, healing of the wound always begins near the cut conductive tissue of the phlowm and that, in the union of graftedparts of plants, the close connection of phlowm tissues is essential. But as yet studies of the hormones of cell division are far less comp;ete that those of the growth-promoting tissue hormones.

On the basis of the hormone hypothesis Swingle (1926) has explained the fact that date pollen influences not only the character of the date seed, which may be explained by xenia, but also influences the size, shape, character, and time of ripening of the fruit. He believes that the embryo or endosperm of the date seed, or both together, constitute a ductless gland apparatus which by secreting hormones affects the development of the entire date fruit. Montemartini (1929) found that when a red-hot wire was thrust into an unripe pear, penetrating and killing the seed, the ripening of the pulp was greatly accelerated. This also is interpreted as the effedt of a hormone which diffused out form the seed into the fruit.

Likewise Went (1926) has explained the phototropic response of oat seedlings as due to a photoreactive hormone which is produced at the tip of the coleoptile and descends on all sides. Its function is to increase the elasticity of the cell walls and permit the cell to increase in size (grow). "Light checks the descent of the hormone, so that when illuminated even an extremely small amount, the hormone passes down only on the shaded side, where growth then occurs, with the result that the plant bends towards the light. The hormone is thus seen to be a "growth hormone" rather than a "trop-hormone."[1] These results have been confirmed by Soding (1925-1929) who also found that when decapitated coleoptiles were reattached by a gelatin bridge, growth was more rapid than when the tips were not replaced. When peduncles of Cardamine and Cephalaria were cut off and reattached by agar, similar results were observed, which indicate that there is some substance, i.e., a hormone, which can cross the agar or gelatin bridge and affect the growth of the parts below.

In conclusion, the hormones are thought to stimulate the action of enzymes, which in turn bring about the metabolic changes we find in plants. The hormone is thus the "trigger" which excites enzyme activity and causes the enzymes to be secreted. Similar results in the formation of galls and other excrescences are produced by the injection of materials when the plant is stung by gall insects, and thyroid extract has been found to promote

1. Raber, Principles of Plant Physiology, (New York, 1933) Page 210

enormously the growth of hyacinth bulbs and potato tubers. How the hormones bring about these changes is still unknown, but Denny (1926) found that the dominance of the apical bud in potato tubers can be checked by thioures; in place of only one, two to five buds then develop from each eye. It has been suggested that hormones change the permeability of the protoplasm thus causing changes in the osmotic conditions inside the cell. Others have proposed that they cause a separating out of the consituents of the protoplasm, much as freezing might, and in this way aid in secretiry activity. The complete chain of chemical reactions still remains to be worked out; only a few links have thus far been forged. Plant hormone research is just beginning and there is a bottomless pit yet to be explored.

PART II --- PHOTOTROPIC RESPONSES

No phenomenon of plant life is more familiar than the turning of leafy shoots toward light or the orientation of leaves in a manner to occupy a favorable exposure. Plants placed at the window of a dark room promptly show the effects of the light stimulus. The same relations may be observed in the field. The capacity to show through growth curvatures an irritable response to light from one side is called phototropism. We have to distinguish as main classes of responding structures those axes which are parallelotropic, curving in such manner that the tips point toward or away from the source of light, and those whichare plagiotropic, or at some angle. Leaves are transversely phototropic, and the response secures a favorable illumination of the chlorophyll bodies. As a result broad-leaved plants develop commonly to form a more or less perfect mosaic, no better examples of which can be found than those of the grape-vine or Boston ivy.

Light Perception:

Phototropic organs may possess special perception regions and these regions do not necessarily correspond to those of curvature or bending. The method of perception id not understood, but the sensitiveness of the mechanism is almost incredible.

Influence of Light upon the Direction of Growth of Organs:

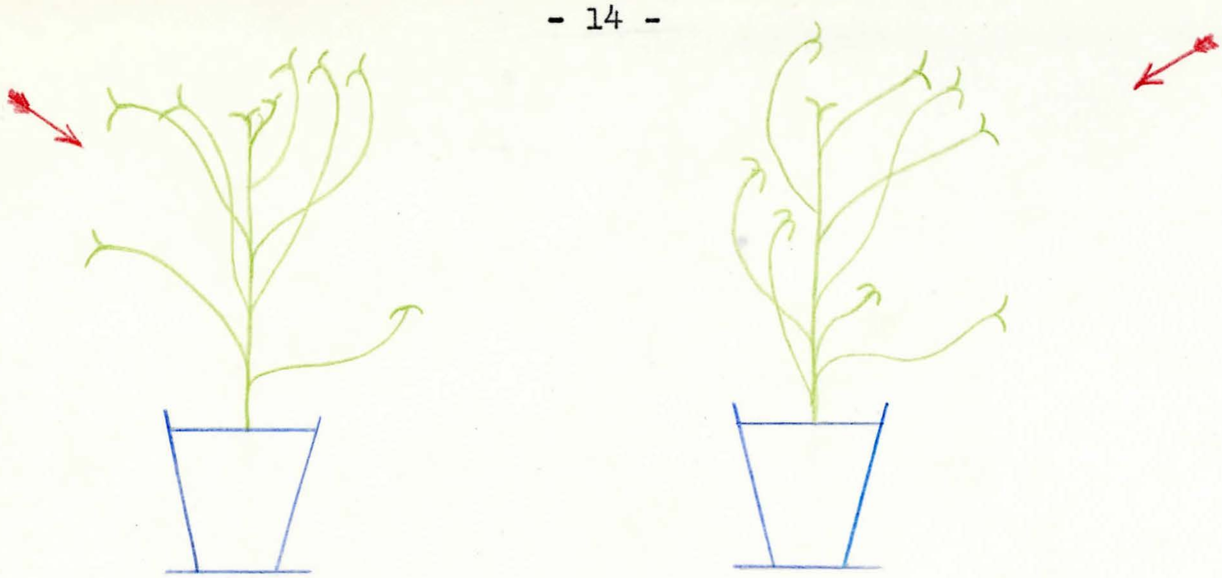
Light is one of the factors indispensable to the life of green plants, providing them with the energy required for the decomposition of carbon dioxide. It is but natural therefore that plants should display a high degree of sensitivity in relation to light and should respond effectively to changes in the direction and intensity of the light rays falling upon them. The ability to react to the directive influence of light is called "phototropism". Young growing stems usually curve toward the source of light such a reaction being termed "positive phototropism". Curvature in the direction opposite to incidence of light is "negatively phototropism", while the capacity of adjusting organs, e.g., leaf blades in a direction perpendicular to the incident rays is known as "diaphototropism".

Not all the organs of a plant are phototropically sensitive. This is a characteristic feature only if the aerial parts of the plant. The underground organs, such as roots and rhizomes, which normally grow in darkness, very often do not react to light. Some roots, however, like those of mustard and other crucifers, are negatively phototropic.

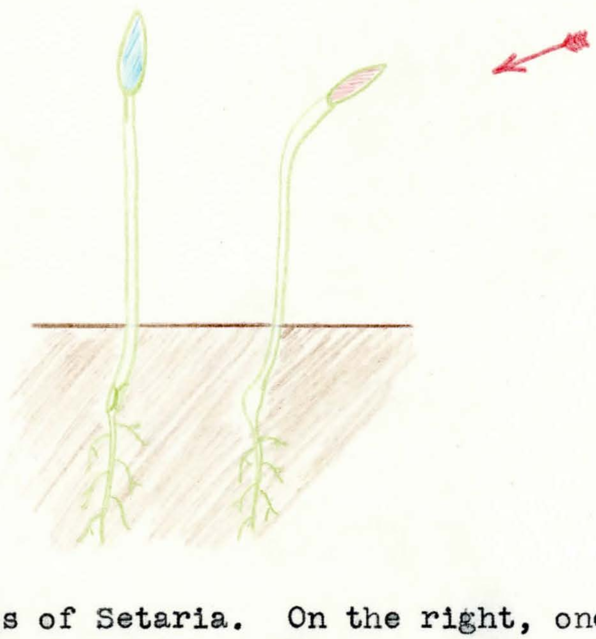
The capacity of bending toward or away from light is not permanent in every organ. It depends on the intensity of light. In too strong light, positive curvatures frequently change to negative ones. By selecting various intensities of light, it is possible, therefore, to make the same shoot bend in succession toward and then away from the source of light. It is also possible to find a certain intensity of light in which the positive effect will be neutralized by the negative; the plant will then appear to be insensitive to light.

Phototropism plays an important part in the life of plants. On account of it, leaves, which require light, assume the position that is most favorable for the utilization of this source of energy. They usually spread perpendicular to the direction of the incident rays. Owing to phototropic movements, the leaves of many plants form the so-called "leaf mosaic", which is particularly conspicuous in plants growing in shady places.

Phototropic sensitivity is in no way connected with the presence of chlorophyll. Sporangia and the fruiting bodies of many colorless fungi also manifest phototropic curbatures. Moreover, etiolated seedlings are always more sensitive to light than green ones. In general, it has been found that the growing of plants in light decreases their phottotropic sensitivity. The seedlings of some cereals, particularly those of sorghum and millet are most convénient objects for such experiments. These seedlings are of a rather consistent character composed of a rather long hypocotyl bearing a comparatively small coleoptile, which encloses the first true leaf. If the shoot is placed in a phototropic chamber, the hypocotyl will form a distinct curve, and the tip of the coleoptile will be directed toward the light. At first the curvature is formed directly beneath the tip of the coleoptile, but later, as further growth of the hypocotyl takes place, the curvature is in a somewhat lower region. By means of simple experiments, it may be easily shown that the coleoptile is the organ that perceives light, while the hypocotyl merely responds to the stimulus transmitted from the coleoptile. If an opaque hood made of black paper or tin foil is placed over the tips of the shoots so as to cover the whole of the coleoptile, no curvatures are formed, notwithstanding the fact that the hypocotyl remains exposed to light. On the other hand, if the hypocotyl is shaded from light and only the coleoptile remains exposed to it, curvatures are formed as distinctly as when the whole of the shoot is exposed.



A geranium adjusting its leaves perpendicular to the direction of light (after Lubimenko).



Seedlings of Setaria. On the right, one bent toward the light; on the left, one of the seedlings has been left upright. Its plumule has been covered with a non-transparent cap (after Holman and Robbins).

"The mechanism of phototropic curnatures consists in a more retarded growth of the exposed side of the stem as compared with the growth of the shaded side; thus the lighted side becomes shorter, and the entire organ will curve."[1] Comparing this fact with the long-known phenomenon of delayed growth caused by the effect of light, many authors, beginning with De Candolle attributed this bending of plants toward light to the direct retarting affect of light on the growth of the exposed side. But considering the experiments that have demonstrated that perception is localized in the tip of the shoot which is not directly subject to curving the foregoing explanation has to be modified as follows: A unilateral exposure to light causes certain substances to appear in the organ of perception; these descend to the growing zone and produce a different effect upon the growth of the exposed and the shaded sides.

If all the data concerning the physical nature and the manner of the transmission of phototropic stimuli are correlated with the dependence of the rate of growth on the amount of the growth hormone manufactured by the stem tip or the coleoptile, the following mechanism of phototropic curvatures may be observed. Under the influence of the stimulus of light, the total amount of the growth hormone in the sensitive apex, as well as its distribution is altered. As the exact quantitative determinations by Went have shown, light in itself decreases the amount of the growth-promoting substance in the apex. This explanationSatisfies the retartding influence of light on growth. Moreover, under the influence of unilateral illumination, there takes place an electrical polarization

1. Maximov, Plant Physiology (New York, 1938) Page 154.

of the cells that produce and transmit the growth-promoting substance. The illuminated side receives a negative, the shaded side a positive, charge. Under the influence of this polarization, the current of growth-promoting substance is shifted to the shaded side. The cells of this side elongate more repidly and more strongly than do those of the opposite side, and as a result a curvature of the seedling toward the light appears.

Not all the rays of the solar spectrum produce an equal phototropic effect. Red rays, as a rule, are least effective. Toward the blue end of the spectrum, there is an increased effect, which attains its maximum in the indigo-blue rays (4650A Units) and then again gradually decreases toward the ultrviolet region.

Interesting studies have been made upon the use of artificial light in greenhouse culture. as in forcing lettuce, radish, and certain flowers. In such experiments the artificial light has been employed usually at night, or supplementary to daylight. Economically, artifical light is probably a failure, owing to the expensiveness of it; but the results of the wxperimental work bring out some points of interest.

Many experiments have been made to determine appeoximatel the effects of light of different wave lengths on the form and structure of plants. In much of the work which has been done pure screens were not employed, yetthis type of work is sufficiently important to justify careful physical methods. In general, the dry weight of plants grown for a considerable period under monochromatic screens is greatest in red, and least in violet; yet the growth in red is not equal to that in white light. The violet rays are also important in the production of bloom.

In the following table there are given, after Teodoresco, the relative areas of leaves developed from the bud in different qualities of light during a period of about thirty days. With each plant the leaves occupied equivalent positions on the young shoot: --

Plant Employed	Kind of Light				
	White	Red	Green	Blue	Darkness
Vicia Faba	948.3		127.8	654.8	52.8
Lupinus albus	158	49.5	38	62	8
Polygonum Fagopyrum	128	59	23	64	11
Ricinus sanguineus	1105	503	200	600	53

Effect of Wave Length upon Area of Leaves, Area in sq. mm.

Heliotropism:

The term heliotropism is often used in place of the word phototropism. Heliotropism is the orientation of plant organs in response to sunlight. Stems and leaves, however, respond to artificial light as well as to sunlight, so that it is better to use the general term, phototropism, than the specific one, heliotropism.

Etiolation:

Stems that grow in the dark have a tendency to grow longer and to be more slender than those that develop in the light. They have a blanched appearance, due to a lack of chlorophyll. The leaves of plants grown in the dark are usually small and also have a blanched appearance. Plants that stems and leaves with these characteristics, which are the result of growth in the dark, are said to be etiolated. Etiolation is well illustrated in experimental work with the Balsam Apple.

In nature, when upright stems grow in the dark they usually arise from underground structures such as bulbs or rhizomes, or are produced by germinating seeds. In such cases the relatively longer, slender structure of etiolated stems has a tendency to make the plants reach up into the light. The production of small leaves on etiolated stems also seems to be of advantage, as leaves in the dark cannot carry on photosynthesis and so are of no particular use to the plant. Moreover, large leaves would hinder the growth of stems through the ground, while their formation would require material which could be usedin elongating the stem.

PART III --- CONCLUSIONS

This part of my paper is more or less intended as a general survey of the work done and the preparation of a series of conclusions.

Conclusions derived from experimentation and reading:

1. The tendency of plant organs to orient themselves with reference to light is called phototropism.

2. Nearly all roots that normally grow in the groun show little or no phototropism; but there are some exceptions, as in the case of the radish, where the roots are negatively phototropic.

3. In coleoptiles it is the tip that is sensitive to light, while curvature occurs at the base. There is thus a region of perception of the stimulus at the tip which is separated from the region of response at the base.

4. In the stimulated tips of coleoptiles and roots there is formed a growth-regulating substance or hormone, and it is the diffusion of this hormone toward the responding region that results in the unequal growth which produces curvature.

5. The existence and diffusion of a growth-regulating hormone has been demonstrated in several ways. The tip of a coleoptile or root can be cut off and separated from the remainder of the coleoptile or root by gelatin, when the hormone diffuses through the gelatin to the stump of the coleoptile or root and produces curvature. If a

sheet of tin foil is placed between the tip and the remainder of the coleoptile or root no response results.

6. There is an indication that only one growth-regulating hormone is concerned, as the tip of a coleoptile which has been stimulated can be cut off and placed on the end of a root from which the tip has been removed and cause curvature.

7. A gelatin block into which the hormone has diffused from an stimulated root tip will cause curvature in a coleoptile.

8. Auxin, a growth hormone, is a monobasic acid of the empirical formula, $C_{18}H_{32}O_5$.

9. The growth hormone is capable of moving through the plant only on one direction, from the proximal end toward the base, and never moves in the reverse direction from the base to the top.

10. Auxin produces an accelerating effect only upon the stage of elongation and does not influence the division of cells, which determines the stage of meristematic growth.

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