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INTERNAL FACTORS OF PLANT FLOWERING[1]

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The individual development or the ontogenesis of plants, which begins with the ovary's fertilization or with formation of an embryonic bud and ends with death, comprises the whole cycle of the plant's life and includes all its life processes. The transition of plants from vegetative growth to flowering, when new progeny develops in tissues of the maternal plant, is the most essential period in ontogenesis. The physiological nature of the transformations which precede and condition the formation and development of floral organs is the main subject of this paper.

The morphological picture of flower development, outlined in the theory of metamorphosis by the great poet and botanist Goethe, who thought of a flower as a transformed leaf shoot, is now widely recognized and has been developed by numerous scientists (182). The physiology of processes which precede the development of a flower has not been studied as well; the picture is vague and contradictory though the earliest conjectures go back as far as the second half of the last century. And if anybody were asked to name a phenomenon of plant life where the gap between morphology and physiology is the widest, he would certainly indicate flowering.

Sachs's hypothesis (162) on plant-forming and especially on flower-forming substances, which was first published in 1880, was the earliest concept of the physiological nature of plant flowering. Klebs's theory (95) of the role of the ratio of nutrients appeared much later (in 1918). A new phase in the study of the physiological nature of the flowering phenomenon began immediately. In 1918, Gassner (70) found that winter forms require cold and that they differ in this requirement from spring forms; he discovered the phenomenon of thermoinduction in plants while in 1920 Garner & Allard (69) discovered photoperiodism in plants.

During the half century that has passed since that time, the physiology of flowering became enriched with new basic data and with essential theoretical conclusions. These findings have been reviewed in articles regularly published in the *Annual Review of Plant Physiology* (56, 62, 105, 112, 116, 165, 172), in detailed reviews published in the *Encyclopedia of Plant Physiology,* Volume 16 (119, 139, 140, 153), as well as in other articles and books (34, 76, 86, 107, 166, 197, 204). The present state of the problem has been fundamentally outlined by Lang (109) in the *Encyclopedia of Plant Physiology*.

[1] The survey of literature pertaining to this review was completed in November 1966.

This paper is not a critical review of publications on flowering physiology for a restricted period; it discusses principal trends in the investigation of the role of internal factors which prepare the transfer of plants from vegetative growth to flowering.

Environmental and Internal Factors

Plant flowering, which takes place during the transfer of plants from vegetative growth to reproductive development, like all natural phenomena, occupies a certain position in time and space and has a certain material content. The study of the process of flowering is, therefore, connected with the solution of three basic problems: (*a*) when flowering takes place; (*b*) where and in what parts and organs; and (*c*) what phenomena and what physiological transformations lie at its base.

An answer to the first question can be found in the realm of phenomena where both genetic factors and plant reactions to environmental conditions play a decisive role. Under common natural conditions, flowering of seed plants takes place after the formation of vegetative organs or, more precisely, after they have passed the young age or juvenile stage. The duration of the juvenile period in many plants depends on their sensitivity to photoperiodic and thermal induction (60, 88, 100, 168, 206), the minimum number of leaves at the moment of flowering varying from 3 to 4 in *Pharbitis* to 37 in *Eupatorium adenophorum* (87, 103). The duration of the juvenile period in the diversity of the world's flora averages several days in such ephemeral species as *Arabidopsis thaliana* or *Baeria chrysostoma* (103, 193) and several decades in arborescent giants of the plant kingdom.

Genetic factors find their expression in the very division of plants into perennial and annual, winter and spring, late and early. In addition to genetic factors, physiological factors, i.e., the reactions of plants to the changing of environmental conditions which are equally typical both of perennials and annuals are also of the utmost importance. However, physiological factors are not as evident in perennial plants as they are in annual plants. For instance, olive trees (*Olea europaea*) start flowering at low temperatures (80); coffee trees (*Coffea arabica*) form flowers under short-day conditions (193); *Cestrum nocturnum* flowers with the successive alternation of long and short day (163); perennial herbaceous plants flower under more complicated environmental conditions; and, finally, biennials have reactions which are similar to those in annual winter forms. The juvenile period of the ontogenesis of perennial tree species is the least susceptible to environmental control; the explanation lies in transformation of physiological and structural age which conditions their conservatism with regard to flowering and fruiting dates (63).

A large role in the onset of flowering in annual and perennial plants is played by reactions to the day length, i.e., photoperiodism and to lower temperatures, i.e., vernalization[2] as well as to the intensity and quality of light, moisture conditions, and mineral nutrition. All of these reactions are adaptative properties for survival under unfavorable weather conditions in different seasons of the year. Beginning with research by Lubimenko & Scheglova (118), it was shown that by and large, short-day species inhabit tropical and subtropical areas, long-day species moderate and northern areas, and day-neutral species quite different latitudes. It was also shown that the adaptation of plants to day length is of a more varied nature because it is related to seasonal changes of other environmental conditions especially to temperature and humidity (176). Thus the day length, as an astronomic phenomenon, has proved to be the most reliable and accurate signal informing plants when they must flower and propagate and when they must be ready to survive unfavorable environmental conditions.

We now know seven photoperiodic groups which differ in their response to day-length conditions (109); in addition to long-day, short-day, and day-neutral plants, there are long-short-day (first long day and then short day), short-long-day (first short and then long day), medium-day or stenophotoperiodic (intermediate day length), and amphiphotoperiodic (only long day or only short day but not intermediate day length). This classification is especially expressive with regard to the adaptative nature of the reaction of plants to the day length, which is more general in long-day and short-day species and more specialized in other species, for example, in long-short day species which respond to the changing day length of the summer-autumn season, and in short-long-day species which respond to the changing day length of the spring-summer season (55, 64, 157, 161, 185).

Maximov (126) and other authors have found that the development of winter forms is an adaptative property for plants which overwinter. A manifest division of plants into winter and spring forms is observed in areas with clearly observable division into winter and summer; this division is absent in areas without winter and summer. That is why winter forms are not found among day-neutral and short-day species, being observed only among long-day species which adapted to day length and to lower temperatures at the same time. The adaptation to the effect of lower temperatures varies a great deal, as it does in the case of photoperiodism, so there are winter forms which possess different degrees of winter disposition depending on the duration of the effect of lower temperatures and on their level. There are strict correlations between the winter disposition of species and the natural conditions of the winter period at their growing site (156).

Besides photoperiodism and vernalization plants possess many other

[2] The term "vernalization" was introduced by Lysenko (115); it is in fact the thermoinduction of winter forms and seedlings of biennials provoked with the help of lower temperatures during a certain period. Thermoinduction corresponds to photo- or photoperiodic induction.

adaptative reactions, among them reactions to light intensity and spectral composition, mineral nutrition, soil moisture, air humidity, etc.

Spring, day-length neutral, and early-maturing plant species go to flowering in an orthodox manner, without the interference of any adaptative reactions. At the same time internal factors of flowering as a turning point in the ontogenesis of plants have been studied for more than forty years mostly on photoperiodically or thermally sensitive plants; these studies are always closely connected with the investigation of photoperiodism and vernalization. They depend on the fact that flowering in spring, neutral, and quick-maturing species is a rapid phenomenon, and difficult to study. As to flowering of long-day and short-day species, spring, winter, and winter vernalized forms, it is an easily controlled process which can be obtained at a desirable period.

The use of photoperiodism and vernalization to study flowering processes can be compared with slow filming of sprinters who quickly cover short distances or gymnasts who spend but a minute to make difficult gymnastic exercises.

Interaction of Organs

The study of internal processes in flowering resulted first of all in the discovery of those regularities which are connected with the interaction of organs in photoperiodic and vernalization processes, and thus it was possible to answer the second question—where and in what parts and organs of plants processes take place which prepare plants for flowering.

The first step in the field of photoperiodism was made by Knott (97) who showed that in long-day rosette species of spinach long days affect leaves and not the apical bud. The proof that leaves and not apical buds are the receptive organs of the photoperiodic effect became even more evident in experiments on short-day species where leaves are spaciously separated from buds—on *Chrysanthemum* by Moshkov (134) and by Chailakhyan (27) and on soybean plants by Psarev (151). These facts were fully corroborated later both for long-day and short-day species.

The susceptibility of isolated (i.e., removed from plants) leaves to the photoperiodic influence effect became the object of a special study. It resulted first in discussion and later in the clear conclusion that leaves of short-day plants, entirely isolated from plants, can perceive the photoperiodic influence effect (5, 35, 203). Isolated leaves of long-day species soon aged, lost their turgor, and died before grafting; in this connection another procedure was tried; in one case, buds were removed from rosette plants of *Rudbeckia* during exposure to long-day conditions and in another case, roots. In both experiments, however, the leaves of the rosettes proved photoperiodically sensitive (37).

As a result of the photoperiodic effect, leaves become physiologically transformed, these transformations being of a biophysical and biochemical nature. Numerous experimental data testify that these transformations result in the formation of certain metabolites, some of which, under favorable day-length conditions, are necessary for flowering.

The formation of floral organs depends not only on metabolites delivered from leaves but also on the physiological state of the apical bud per se. For instance, this state changes if an apical bud is affected by leaves developing under unfavorable day-length conditions (27, 134). There is a certain competition between metabolites moved from long-day and short-day leaves in their effect on the bud, the result of the competition depending on the placement of these leaves in relation to the buds, and after all on the comparative rate of the delivery of these metabolites to the bud. There is no direct interaction between metabolites of long-day and short-day leaves in the stem because the reaction of the bud on a plant with a stem split to the very top with long-day and short-day leaves growing on different parts of the stem was the same as in whole (unsplit) plants (29). Flowering of rosettes of long-day plant species is also affected by metabolites synthesized by roots because these plants, when deprived of roots and exposed to long-day conditions, are unable to form stems and do not flower (30).

Thus it has been found that flowering is the result of the functional activity of all three main organs—leaf, stem, and root. Leaf metabolites are photoperiodically related and determine the start of flowering; metabolites of apical stem buds affect the rate of flowering, stimulating, or inhibiting it; and root metabolites of long-day species determine the growth of the stems on which flowers are formed (31).

It is known that seedlings of winter forms and biennials sown in spring and grown under conditions of optimal nutrition are unable to perceive the photoperiodic stimulus as a factor which regulates their development; they remain entirely indifferent, that is, "blind" to day-length changes. Only plants exposed to the effect of lower temperatures for a certain period have photoperiodically sensitive leaves. The reaction to the effect of lower temperatures of germinating seeds of winter forms is concentrated in embryos, the remaining portion of seeds being a source of substances which are necessary for this process (72). In seedlings of the same forms and biennials, this reaction develops in tissues of apical stem buds (128). Thus apical stem buds are the receptive organs, i.e., organs for the perception of lower temperatures and not leaves as is the case with the photoperiodic effect. Metabolites which are formed in buds are specific for vernalization; they move to leaves and roots and provoke corresponding physiological transformations.

The outlined picture of the interaction of organs at the start of flowering is only a part of the many-sided interrelationship of physiological processes which is always present in plants. Just as in animals whose processes are integrated so that the nervous-humoral system may be considered independently, along with circulatory, digestive, and other systems, the plant's hormonal system should be considered as different from other systems which provide energy and nutrition functions, providing for integrity of the plant's vital processes (31, 102). The interaction of organs in flowering processes and the formation of the organs of vegetative propagation begins as an organism's response to environmental effects; it develops as a result of the irritability which plants and animals possess (74). This responsive reaction consists of the following phases: (*a*) the perception of the external stimulation by a receptive organ; (*b*) the transformation of the physiological state of the receptive organ and the formation of specific metabolites; (*c*) the translocation of metabolites to other organs and parts of plants and (*d*) changes in the physiological state and morphological transformations in these latter organs and parts. These phases strikingly coincide with long ago discovered phases of tropic reactions: (*a*) stimulation; (*b*) irritation; (*c*) transmission; and (*d*) reactivity (31).

All this testifies that the responsive reactions of plants to environmental conditions, with four successive phases, conform to a general regularity which characterizes the interrelation of plants with environment.

Trophic Factors

Klebs's theory of the role of nutrients and Sachs's hypothesis of the role of formation substances in plant flowering were based on quite opposite ideas; these two scientists initiated two trends which were followed in further studies on the internal mechanism of plant flowering and which developed independently without any attempt to unite them into a single concept. However, recent data and conjectures became so significant that it has become possible to sum up the results of these two trends, to show stable relations between trophic and hormonal factors of plant flowering, and at the same time to approach a solution to the third question of what lies at the base of plant flowering.

Investigators of this complicated problem used both analytical methods —analytical determinations of the content and dynamics of changes of this or that substance in plants induced or not induced to flowering—and synthetic methods—the study of the effect of these or similar substances introduced into plants from outside. Only when the results provided by these two methods coincided could one picture the participation and role of certain substances in plant flowering.

In the field of the study of trophic factors in flowering, studies of conditions of light and root nutrition and of photoperiodism in plants are of the greatest importance.

Photoperiodism is so strong and so reliable an instrument of the control of plants' growth and development rates under laboratory conditions that internal factors of flowering and photoperiodism have always been studied with an unbreakable integrity.

Nutrition and carbohydrate-nitrogen metabolism.—The role of nutrients in flowering—sugars formed in the process of photosynthesis and nitrogenous compounds introduced through plant's roots—was considered of primary importance in Klebs's theory. This theory was based on many years of well designed experiments which showed that all conditions essential for flowering are also favorable for photosynthesis and consequently for the accumulation of sugars, while the intensive mineral, especially nitrogenous, nutrition stimulates vigorous vegetative growth and inhibits flowering. Klebs's theory was soon energetically supported by many scientists, and it was applied to plant growing and horticulture where its use became fruitful in connection with the application of fertilizers, frameworking, and ringing of fruit crops. However, it did not stand unchallenged for long. Soon after the discovery of photoperiodism, it was found that the start of plant's flowering under different day-length conditions contradicted his principal concept.

It was found that all plant species despite the nature of their photoperiodic reaction contain more carbohydrates, i.e., sugars and starch under long-day conditions and more nitrogenous compounds and proteins under short-day conditions. This means that there is no definite correlation of carbohydrates and nitrogenous compounds at the start of flowering of all these species. At the same time experiments on the application of nitrogenous fertilizers to a substrate showed other regularities of their effect on flowering. It was found, for instance, that the deficiency of nitrogen in nutritive substrates stimulates flowering of long-day species like barley, oat, mustard, and others, and inhibits flowering of short-day species like millet, corn, tobacco, chrysanthemum, and others; a surplus of nitrogen, on the contrary, stimulates flowering of short-day species and inhibits flowering of long-day species.

Thus there is a regular correlation between the nitrogen-floral reaction, as the reaction of plant flowering to nitrogenous nutrition is called, and the photoperiodic reaction, with new data corroborating the existence of this correlation (160, 173).

The opposite reactions of short-day and long-day species were provoked by the artificial introduction of saccharose solutions into plants through peduncles or by infiltration into leaves: this resulted in the sharp inhibition of flowering in a short-day *Perilla* species and in the stimulation of stem growth and either stimulation of the formation of floral organs or no effect in long-day henbane and *Rudbeckia* species. So the experiments on the application of nitrogenous fertilizers to substrate and on the artificial introduction of sugars into plants show that flowering of long-day species takes place with nitrogen deficiency and carbohydrate surplus while flowering of short-day species, on the contrary, is stimulated by abundant nitrogen and comparatively low carbohydrate content. However, further approaches to the discovery of relations between the qualitative composition of amino acids and proteins and flowering processes have not yet resulted in any definite conclusions (57, 89, 125, 130, 142). Changes of phosphorus and potassium content in the substrate affect flowering to some extent but their effect is less significant and is not connected with the nature of photoperiodic reaction (66).

The comparison of data shows that in metabolism that precedes the formation of floral organs the carbohydrates-nitrogen ratio does not condition the start of flowering at all, but it is of certain importance and can be considered a system related to the metabolic preparation for flowering.

Light reactions of photoperiodism, photosynthesis, and photochemical processes.—The photoperiodic process of flowering consists of successively developing light and dark reactions. Light and dark reactions, day and night, are of great importance for photoperiodism, so it would be more correct to call the process photonictiperiodism (42). However, the term "photoperiodism" has become universally recognized; it is tightly fixed in history.

There are two types of light reactions: (*a*) reactions which take place only with high-intensity light, and (*b*) reactions which take place with light of both high and low intensity. It has been found that both in short-day and long-day species photosynthesis is the first important stage that passes during the main light period (8 to 12 hr) of the daily cycle under the conditions of high-intensity light, presence of carbonic acid, and sufficiently high temperature. However, this process is not specifically for photoperiodism; it serves only as a source of assimilates. Such concepts have been suggested earlier too; now there are accurate experimental data that convincingly testify that photoperiodic reactions take place also when photosynthetic products are artificially substituted with different sugars, organic acids of the Krebs's cycle, reduced ascorbic acid, cysteine and glutathione (20, 68, 107, 117, 121, 141).

Three other groups of facts also testify that photosynthesis is important in photoperiodism as a process that delivers initial materials for further transformations: (*a*) the ability of plants of different photoperiodic groups to flower under conditions of constant darkness both when they are grown from seeds and after transfer of big vegetative plants from the unfavorable day length [this ability was observed in numerous studies, some of them quite recent (146, 183)]; (*b*) the ability of plants to flower within the wide range of chlorophyll content, equally manifest in green (44, 73), chlorotic, etiolated, and even albino plants provided with sugars (82, 169, 181); and (*c*) the photoperiodism of animals, which have no photosynthetic ability, widely spread among various groups and having many features in common with photoperiodism in plants (81).

Subsequent photoperiodic stages are: in the long-day 24-hour cycle—an additional light period during which respiration and light reactions are observed—and in the short-day cycle—a dark period during which respiration processes and dark reactions are observed. The transformation of assimilates during these periods and the resulting metabolites are specific and condition the accomplishment of photoperiodism connected with flowering.

Reactions to an additional light period in the long-day cycle take place in the absence of carbonic acid and in the light of a wide range of intensities—from sun to moon light. The quality of light is of decisive importance; however, until recently there has been no unanimous opinion with regard to the role of monochromatic light during the additional light period. According to some studies orange-red rays actively stimulate flowering of long-day plants and oppress flowering of short-day plants, blue-violet rays being the least active (96, 104, 119). Other scientists, on the contrary, have shown that violet, blue and far-red rays are the most active while green and red rays are the least active (131, 179, 190).

The contradictory nature of these data does not deny the general conclusion that light of different quality, acting during the principal light period of the short day, does not affect plant development because the process of photosynthesis predominates there; as to long-day conditions, light of different quality produces the same effect whether it is applied during the whole light period or only during an additional light period. The quality of light during the additional light period displays itself more evidently at low light intensity.

At the same time reactions to the additional light period take place only at sufficiently high temperatures (143, 191, 202) in the presence of oxygen. Under anaerobiosis, flowering of long-day species—*Rudbeckia,* oat and pea —was strongly inhibited or fully stopped while flowering of short-day species—soybean, millet and cocklebur—was stimulated (19, 50).

All these data corroborate the conclusion that reactions to the additional light period are connected not with photosynthesis but with the photochemical process which affects induction and with the aerobic respiration which takes place in the light.

Dark reactions of photoperiodism and respiration.—Reactions of the dark period in the short-day cycle have specific features and take place only under certain conditions. First of all dark reactions differ from light ones in that they do not permit interruption while light reactions do permit ruptions. When there is a lasting period of darkness of the short-day 24-hour cycle with short light intervals which last several minutes, flowering of short-day species is inhibited while that of long-day species is hastened. Reactions to the interruption of darkness are similar to reactions to the additional light periods both in their effect and in that they do not require a high intensity of light and are not connected with photosynthesis.

Dark reactions take place within definite temperature limits, the decrease or the increase of temperature inhibiting their development as testified by the reaction of plants: flowering of short-day species is inhibited (119, 135, 137, 170, 191) and that of long-day species is hastened (99). Dark reactions, however, require the presence of oxygen because growing plants under anaerobic conditions, i.e., in an atmosphere of nitrogen (50, 177, 201), chloroform, or in an atmosphere enriched with carbonic acid are inhibited or suppressed by them (19). The most serious inhibition of dark reactions occurs after a change in the temperature regime or when anaerobiosis is imposed during the second half of the darkness period. The dependence of dark reactions on temperature and on the presence of oxygen shows that these dark reactions require aerobic respiration (98).

When separate links of the respiratory process are suppressed by keeping cotyledons of *Ipomea* (or *Pharbitis nil*) in the solutions of inhibitors —azide, cyanide, and fluoride (138)—or of *Perilla* and *Rudbeckia* leaves in cyanide vapours (3), flowering of short-day species was suppressed and that of *Rudbeckia* was stimulated. Flowering of cocklebur was also suppressed when cocklebur leaves were treated with dinitrophenol solution which uncoupled oxidative phosphorylation (164). This suppression was observed mostly under the exposure of plants during the second half of the dark period; and in cocklebur leaves, treatment with cobalt ions inhibited flowering only during the first half of the dark period (165); it was then removed by the application of ascorbic acid, cysteine, or glutathione.

Any direct connection of dark reactions with protein metabolism was not found because a specific inhibitor of protein synthesis—chloramphenicol—did not inhibit cocklebur flowering (58). Experiments on numerous amino acid analogues showed that only ethionine (an inhibitor of the transmethylation process) inhibited cocklebur flowering during the dark period, this inhibition being removed only with methionine, a donor of methyl groups (7, 165); however, the removal of the inhibiting effect of ethionine with methionine in another plant—*Ipomea*—was not observed (125). The suppression of flowering in *Streptocarpus wendlandii* with ethionine did not result in a change in the content of protein fractions as compared with flowering plants (85). The synthesis of nitrogenous compounds and proteins is probably connected not only with dark reactions but also with the ratio of dark and light reactions of the 24-hour short-day cycle, because under short-day conditions there were the relative increase of the content of nitrogenous compounds and changes of amino acid content (89, 130).

The direct study of the connection of photoperiodism with respiration shows that the higher sensitivity to day-length changes is observed not at the earlier stages of the respiratory process but at its last phase connected with the activity of terminal oxidases. In Axenova's studies (2, 47), infiltration of sodium azide into leaves with subsequent measurement of the respiration rate by oxygen uptake showed that under long-day conditions both groups of plants [long-day species (*Rudbeckia* and oat) and short-day species (soybean and millet)]display an increase in the activity of inhibited respiration caused by metal-containing oxidases while under short-day conditions both groups of plants display an increase in the activity of residual respiration. The consistency with which this ratio of activity of metal-containing and residual-respiration oxidases changes as a result of a change in day length, even during short periods, allows us to assume that the varied nature of respiration is specific for plants grown under long-day and short-day conditions. It means that the ratio of the activity of oxidases containing heavy metals and oxidases of residual respiration can be considered as the second interrelated system of metabolism connected with the induction of flowering.

Vernalization and metabolism.—The reaction of germinated seeds of winter forms, seedlings of winter forms, and biennials to lower temperatures takes place only on the condition that there is sufficient moisture content and oxygen; this proves the importance of oxidation-reduction processes for vernalization. The obligatory presence of sugars delivered to an embryo from endosperm or introduced from outside to embryos separated from caryopses shows that these substances are basic for the whole chain of transformations in which the fermentative processes play an essential role. Metabolites that are formed by vernalization are labile; they are destroyed by high temperatures while moderate temperatures stabilize them. Just as photoperiodic reactions begin from the transformation of photosynthetic products—sugars and other reduced compounds—thermoperiodic reactions also begin with sugars stored in seeds or plant tissues (1, 153).

The principal difference between vernalization processes and photoperiodic processes lies in the fact that vernalization is inherent only in winter forms and in seedlings of biennials while photoperiodism is inherent in a great variety of species, and also in the fact that vernalization is but a preparatory stage to photoperiodic reactions which result in the onset of flowering.

To sum up results of the analysis of trophic factors in flowering, it must be pointed out that flowering of long-day species (under long-day conditions) takes place on the condition that the stimulation of carbohydrate metabolism and higher intensity of that part of respiration that is connected with the activity of metal-containing oxidases occur: flowering of short-day species (under short-day conditions) takes place on the condition that the stimulation of nitrogen metabolism and higher intensity of residual respiration occur. However, two interrelated systems: 1) carbohydrates–nitrogenous compounds and 2) metal-containing oxidases–residual respiration oxidases, exert only a quantitative regulating effect on plant flowering. Hormonal factors and those interrelated systems based on the dynamics of changes of physiologically active compounds are of decisive importance in this process.

Hormonal Factors and Physiologically Active Compounds

Sach's concept (162) of flower-forming substances was destroyed by the first experimental approach; the author failed to show that vegetative growth and generative development are different processes, and could not discover those specific conditions which are necessary to start flowering. That is why Sachs's hypothesis was not supported by later scientists though it had contained a correct suggestion that formation of vegetative and floral organs requires not only nutrients but specific plant-forming substances as well.

There are four groups of hormones and physiologically active compounds presently known in plants: (*a*) auxins; (*b*) kinins and nucleic acid metabolites; (*c*) gibberellins; and (*d*) metabolites which are necessary for flower formation (anthesins). The study of these substances and their antimetabolites or antagonists has contributed much to our knowledge of growth, differentiation, and flowering processes (36, 147, 184).

Auxins.—The discovery of a growth-promoting substance in oat coleoptiles and in canary grass seedlings made by Charles Darwin and his son Francis provided the impetus for numerous experiments on growth-promoting substances and prepared ground for the discovery of natural auxins and synthetic preparations—one of the splendid victories of plant physiology [Boysen-Jensen (11), Went (192), Kholodnyi (92), Went & Thimann (194)].

The initial suggestion that auxins are physiologically polyvalent compounds and consequently can affect not only growth and formation of vegetative organs but flowering as well has proved erroneous because it has been found that the formation of auxins in the top parts of stems of all plants, regardless of the nature of their photoperiodic reaction, is not connected with flowering; it is directly connected with day length, being more intensive in long-day species (59, 94). There are no experiments where treatment with auxin solutions would have resulted in flowering of annual plants under unfavorable day-length conditions. But as soon as the day-length conditions became favorable and provided that there was induction with optimal photoperiods, flowering of long-day species became slightly hastened while flowering of short-day species became slightly inhibited (6, 49, 75, 110, 164). At the same time such growth inhibitors as triiodobenzoic acid and maleic hydrazide have proved able to stimulate flowering and to sharply inhibit growth of short-day species like cocklebur, soybean, and *Perilla*.

These experiments show that auxins and synthetic growth-promoting preparations which function as auxins have indirect influence and do not play a decisive role in plant flowering; they cannot be considered direct regulators of flowering.

Kinins and metabolites of nucleic acid metabolism.—Recently many scientists have paid attention to derivatives of nucleic acids because some of them have been found to influence growth and formation processes strongly and to possess high physiological activity. Purine and pyrimidine bases proved the most active, one of their analogues—kinetin, isolated at Skoog's laboratory (133) from desoxyribonucleic acid of animal origin—being especially active (61). The role of these compounds in vegetative growth processes turned out to be highly significant, though it is much less clear for processes of the transition of plants to a flowering state; however, there are analytical and experimental data in this respect too which show the effect of metabolites and antimetabolites on plant flowering.

Analytical tests showed that leaves of short-day species—soybean, millet, *Streptocarpus wendlandii* and *Chenopodium album*—under short-day conditions contain more nucleic acids than under long-day conditions; the content of nucleic acids in leaves of long-day species varies depending on the day length (16, 71, 84, 189).

Direct experiments on the effect of antimetabolites and metabolites on flowering resulted in data which coincided with analytical evaluations. In all experiments, antimetabolite treatments resulted in the inhibition of flowering in short-day species: in *Streptocarpus wendlandii* treated with 2-thiouracil (84), in cocklebur treated with 5-fluorouracil, 5-fluorodesoxyuridine and 2,6-diaminopurine (7, 71, 142, 164, 167), in *Ipomaea* treated with 5-fluorouracil, 5-fluorodesoxyuridine, 2-thiouracil and 8-azaguanine (125, 204) and in hemp treated with 2-thiouracil (83). It was found that flowering is inhibited either by the direct treatment of buds or after these substances have moved from leaves into buds. Their effect was removed after the introduction into plants of corresponding metabolites—guanine, uracil, thymidine, and orotic acid.

Results of these experiments on the one hand, corroborated the conclusion that plants can start flowering only if meristems of stem buds synthesize a significant quantity of desoxyribonucleic acid (109, 204); on the other hand, they corroborate the concept that flowering is preceded by the synthesis of a specific RNA form which is called "reproductory" in contrast to "vegetative" (84).

The stimulation of flowering was observed in experiments on metabolites of nucleic acid metabolism. Spraying top parts of red-leaved *Perilla* with a dilute solution of kinetin, guanosine, or adenosine solutions at 10 to 12 day short-day induction resulted in flowering while control plants had only begun to form flower buds (49, 123); it was also observed that flowering was hastened in *Ipomea* treated with kinetin, uracil, and guanosine (144). When isolated *Perilla* and *Ipomea* terminal buds were cultivated *in vitro* in White's or Gautheret's nutrient media, results were even more convincing. If kinetin, adenine, guanosine, and adenosine separately, or a mixture of RNA nucleosides (with caseine hydrolysate or without it) were added to the agar substrate of nutrient media, little plants developing from buds started flowering even under long-day conditions while control plants remained in the vegetative state (46).

The difference between experiments on whole *Perilla* plants, where the effect of kinetin was observed only after short-day induction, and on terminal buds, when the effect was observed even without induction, can be explained by the absence of leaves in the second case, as it is known that under long-day conditions the leaves' effect is inhibiting. This suggestion was corroborated as *Perilla* terminal buds grown in White's medium without additional substances under long-day conditions produced rudiments of flowers after a long time (155). The same treatment of buds on whole *Rudbeckia* plants (a long-day species) or of buds isolated and cultivated *in vitro* resulted in the inhibition of stem growth, the retention of rosette form for a long time, and delay of flowering (17, 49).

In animal physiology kinetin is considered a compound typical of the whole group of substances called "kinins." At the same time kinetin strongly affects growth and differentiation of cells, influences the translocation of substances in plants, and inhibits tissue aging (136). Attempts to isolate kinins or, more precisely, phytokinins from plants were successful, 6-N-dimethyl-amine-adenine (named zeatin) being isolated from corn seeds [Letham, Schannon & Mc Donald (113), Miller (133)]. However, there are no definitive data on the effect of phytokinins on plant flowering.

There is no doubt that this group of substances—kinins or phytokinins and metabolites of nucleic acid metabolism—plays an essential role in flowering processes, especially in connection with the role which belongs to desoxyribonucleic and ribonucleic acids in the storing and translating of genetic information.

A comparison of the effect of auxins and metabolites of nucleic acid metabolism shows that they have the same sphere where their effect is the strongest as in meristems of buds. But the nature of their action is totally different: the introduction of auxins results in the stimulation of flowering of long-day species and in the inhibition of flowering of short-day species; the introduction of kinetin and metabolites of nucleic metabolism hastens flowering of short-day species and inhibits flowering of long-day species. It allows us to consider the interreaction of auxins and metabolites of nucleic acid metabolism as the third interrelated system which is important for the metabolic preparation of plants for flowering and which exerts a regulatory effect on the qualitative background of this process.

Gibberellins.—The discovery of gibberellins was a great achievement of plant science because it enabled men to control growth and development of plants [Kurosawa (101); Yabuta & Sumiki, Yabuta & Hayashi (200); Stodola (178); Brian (12)].

Numerous studies by scientists in different countries helped to discover the general function of gibberellins, i.e., stimulation of growth processes in all tested plants, and their specific effects, i.e., induction and stimulation of flowering of many long-day species under short-day conditions but absence of induction and hastening of flowering of short-day species under long-day conditions (15, 33, 54, 106, 122, 124, 198). Thus the chemical induction of flowering in long-day species with the help of gibberellins was obtained, this induction being similar to photoperiodic induction with long days.

The discovery of gibberellins aroused an interest in discovering the physiological nature of vernalization processes. Studies of the effect of gibberellin preparations showed that under long-day conditions they induce stem and flower formation in unvernalized one-year-old seedlings of biennial species—henbane, carrot, parsley, turnip, chicory, cabbage, and European goldenrod (*Solidago virga-aurea*)—as well as in seedlings of winter forms—*Arabidopsis thaliana*, rape, and lettuce (21, 33, 79, 106, 198). In contrast to them the treatment of winter forms of cereals—rye, wheat, and barley—with gibberellins resulted only in the formation and growth of stems and in the slight stimulation of the development of rudimentary spikes but did not induce heading and flowering of these plants (23, 33, 106, 122, 152). Thus, beginning from the first experiments by Lang (106) the chemical vernalization of winter forms and seedlings of biennials was obtained; it was similar to vernalization induced with lower temperatures.

In all these cases gibberellins of microbial origin were used, mainly gibberellin A_3 or gibberellic acid; but soon an idea arose that endogenous gibberellins inherent to plants themselves also participate in the regulation of flowering under certain day-length conditions. The study of the day-length effects showed that in all plants, despite the nature of their photoperiodic reaction, the content of natural gibberellins and gibberellin-like substances was always higher under long-day conditions than under short-day conditions (48, 52, 77, 108, 154), so it is connected with the photoperiodic reaction of growth and not of the flowering of plants. As the period of induction with long-days increased, the content of natural gibberellins in leaves of a long-day tobacco species (*Nicotiana sylvestris*) and a short-day tobacco species (*N. tabacum,* Mammoth variety) became respectively higher. When the same plants and also bean and *Ipomea* seedlings were grown under continuous light, the content of natural gibberellins increased; when they were grown in darkness, it decreased (48, 145, 195) though it had been found earlier in young pea seedlings that the level of extracted endogenous gibberellins decreased upon exposure of the plants to visible light (149). And finally, the interruption of a long period of darkness in a short-day cycle resulted in the stimulation of metabolism and the formation of natural gibberellins; it coincided with the stimulation of flowering of long-day species—*Rudbeckia* and tobacco *N. sylvestris*—and with the inhibition of flowering of short-day species—cocklebur and Mammoth variety of *N. tabacum* (53).

Such similarity of results of analytical evaluations and synthetic experiments testifies that gibberellins play an important role in photoperiodism and the flowering processes. Gibberellins affect the beginning of the formation and growth of flower stems; the result is the beginning of flowering in long-day species which possess a qualitative reaction and the stimulation of flowering under short-day conditions in species which possess a quantitative reaction.

It is interesting that some gibberellins affect flowering while others do not; for example, in an experiment on *Myosotis alpestris* gibberellin A_3 influenced only stem formation while gibberellin A_7 stimulated both stem formation and flowering (132). It is quite natural that such a strong effect of gibberellins on the morphogenetic process is possible only through their effect on plant metabolism (148).

New synthetic organic compounds have been discovered in recent years. These compounds are retardants: they observably inhibit growth and to a lesser extent plant flowering (24). The chemical composition of retardants does not unite them into a single group; they form eight groups of various compounds of which AMO-1618, CCC (chlorcholinchloride) and B-995 are the most widespread (150, 158, 187, 196). Retardants inhibit cell division, retard the growth of stems, and to a lesser extent the growth of leaves and roots, in some cases this effect on growth being connected with their effect on flowering. While the stimulation of flowering was observed in some perennial arborescent species—*Azalea* and *Camellia* (180)—many annual plants treated with retardants were not affected at all or their flowering was inhibited (26, 199). A study of the reaction of plants belonging to different photoperiodic groups showed that long-day species—wheat, henbane, *Rudbeckia,* mustard—were more sensitive to the effect of retardants which strongly inhibited their growth and to a lesser extent their flowering; in short-day species growth was either inhibited (hemp) or was affected to a less extent (soybean, red and green *Perilla*) and there was no effect on flowering (43). The effect of retardants on growth and flowering of plants is quite opposite to that of gibberellins; therefore they can be considered as gibberellin antagonists (120, 187). This conclusion is corroborated by studies which showed that the introduction of AMO-1618 and CCC into the medium blocks the biosynthesis of gibberellins by the fungus *Fusarium moniliforme* and decreases their content in pea seeds (4, 78, 90).

All these studies testify to the idea that endogenous retardants are probably inherent to plants, these retardants being analogues to synthetic ones in their physiological function, and that many growth phenomena which are usually associated with the dynamics of gibberellins should be considered as the function of the balanced action of gibberellins and retardants.

Metabolites necessary for flower formation (anthesins).—Anthesins are hypothetical hormonal substances whose existence is proposed as a result of comparative studies of florigen and gibberellins. Thirty years ago we suggested (28) that plant flowering was connected with substances of an hormonal nature, of flowering hormones formed in leaves which before their classification were known as florigen. This concept was based on numerous experiments, including grafting experiments which showed that: (*a*) typical plants of short-day and long-day species start flowering under unfavorable day-length conditions if they had been grafted to induced plants; (*b*) that short-day plants start flowering under long-day conditions caused by substances formed in leaves of long-day forms; and (*c*) quite the opposite, that long-day species start flowering under short-day conditions caused by substances formed in leaves of short-day species (109). Thus it was found that there are common flowering hormones in long-day, short-day, and day-neutral species.

When it became an established fact that gibberellins induce and hasten flowering of long-day species but do not affect flowering of short-day species, it became clear that they are not florigen. It was then necessary to solve the problem of the interrelation of the florigen complex and gibberellins. Further studies showed that the difference of the reaction of long-day and short-day species to the effect of gibberellins lay in the fact that flowering of long-day species was always preceded by stem formation and growth; while short-day species did not display this relationship as they formed stems both under long-day and short-day conditions. Thus it was found that gibberellins induce stem formation and growth and do not directly influence the development of floral organs. The finding was used as a premise for suggesting that flowering hormones or the florigen complex included two groups of substances formed in leaves: (*a*) gibberellins which are necessary for formation and growth of stems; and (*b*) substances which are necessary for flower formation, conditionally called anthesins (Fig. 1). At the same time it became clear that long-day species do not flower under short-day conditions because of the deficiency of gibberellins while flowering of short-day species under long-day conditions is inhibited because of the deficiency of anthesins (36).

This suggestion was corroborated by experiments where natural gibberellins were extracted with acetone from leaves of *Nicotiana sylvestris* and *N. tabacum* (Mammoth variety), which were grown under long-day condi-

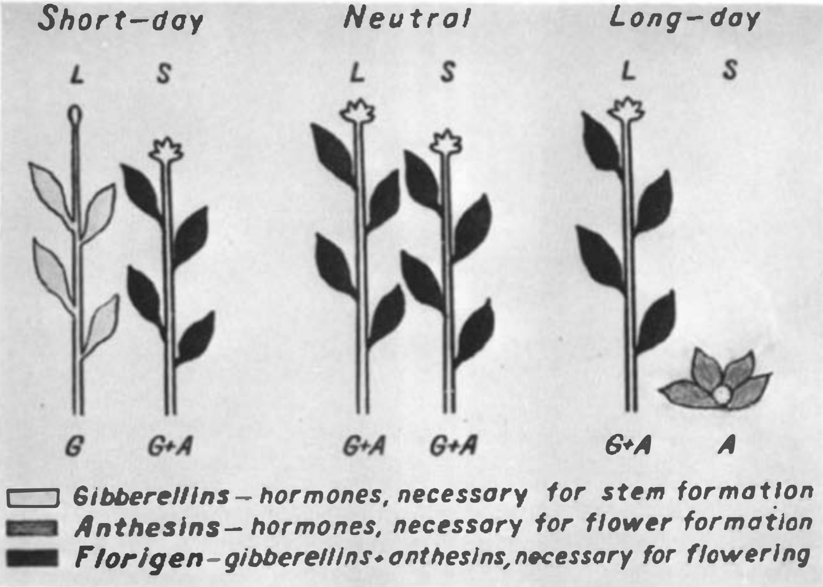


FIG. 1. The scheme of the formation of flowering hormones in various plant species: G: gibberellins (hormones necessary for stem formation); A: anthesins (hormones necessary for flower formation); and G + A: florigen equals gibberellins plus anthesins (necessary for flowering).

tions. Natural gibberellins were then applied by the drop method into rosette *Rudbeckia* leaves exposed to short-day conditions whereupon these plants formed stems and started flowering. In the second case *Rudbeckia* flowering had to be attributed to substances of two vegetative plants—probably gibberellins of Mammoth tobacco formed under long-day conditions and anthesins of *Rudbeckia* formed under short-day conditions (41). In experiments on grafting of biennial henbane rosettes to decapitated vegetating Mammoth tobacco scions, henbane rosettes started bolting, budding, and flowering in contrast to control plants (40). These results fully corroborated earlier data on back-graftings of Mammoth tobacco to rosette seedlings of biennial henbane (129). It is obvious that natural gibberellins in grafted plants exposed to long-day conditions flow from Mammoth tobacco leaves to henbane rosettes and induce their bolting and flowering as is the case when we introduce a gibberellin preparation to henbane rosettes growing on their own roots. Such translocation of natural gibberellins was observed neither in other graftings of the same kind where the same *N. tabacum* (Maryland Mammoth variety) was a donor (long-day conditions) and henbane *Hyoscyamus niger* (annual form) was a receptor (short-day conditions), nor where *Kalanchoe blossfeldiana* exposed to long-day conditions was a donor and *Sedum spectabile* or *S. ellacombianum* exposed to short-day conditions was a receptor (93, 109, 203).

In order to obtain a firm foundation for the concept of two groups of substances necessary for flowering it was considered important to solve two problems. First, to show in graftings of two growing components that flowering of a short-day species under long-day conditions is possible in the presence of substances from the growing long-day species exposed to short-day conditions. Secondly, to isolate anthesins from leaves of a long-day species exposed to short-day conditions and to treat short-day species with them in such a way as to induce their flowering under long-day conditions. These two problems have not been solved though such studies have begun or are already under way.

Among many graftings in which under unfavorable day-length conditions receptors started flowering because of substances from induced donors, there were some in which donors were long-day species grafted to short-day ones, e.g., *Nicotiana sylvestris* tobacco or *Hyoscyamus niger* henbane (annual form)—*Nicotiana tabacum* (Maryland Mammoth variety) exposed to long-day conditions being a receptor; in this case Maryland Mammoth tobacco did not flower but if donors were kept under long-day conditions (grafted plants wholly under long-day conditions), Mammoth tobacco receptors flowered (93, 109, 203). It is necessary to note that grafting experiments, in which two uninduced growing components with the opposite kinds of photoperiodic reactions have been combined, are not numerous, are not enough to settle the above-mentioned problem, and such studies should be continued.

Until recently scientists have not succeeded in isolating substances of the anthesin type which condition flower formation though such work has already begun. Roberts & Struckmeyer (159) reported that they had isolated substances of the lipid type from leaves of flowering cocklebur plants, these substances conditioning flowering in plants of the same species under long-day conditions, but the analysis of their data showed that we were dealing not with the induction of flowering but with the quantitative background of flowering processes. Products of the hydrolytic activity of chlorophyllase, identified as vitamins E and K, were used in strawberry experiments: after the treatment it was found that flowering of plants hastened (175). However, there was no significant difference between the number of flowers from experimental and control plants; the results showed that the substances did not induce flowering but only influenced flower formation. Lincoln, Mayfield, Cunningham, Hutchins, Hamner and Carpenter (114, 127) probably approached the solution of the problem more nearly than others. They took leaves of flowering plants of cocklebur (*Xanthium strumarium*), grounded them, lyophilized and extracted with absolute methanol, and then evaporated the solvent and mixed the sediment with anhydrous lanolin; then they put thin layers of paste prepared by such procedure on the back side of leaves of growing plants of the same species. As a result of such treatment 10 to 30 per cent of the experimental plants displayed the first symptoms of the differentiation of flower buds, these symptoms being observed on sections of tops after 17 to 26 days. Control plants did not display such symptoms. The same results were obtained in experiments on the effect of the methanol extract of sunflower leaves on the formation of flower rudiments in cocklebur (114, 127).

The authors called the active matter from methanol extracts "florigenic acid." We may say about these interesting experiments that the per cent of plants which displayed the effect was low; the reaction was weak; there were no extracts from leaves of vegetative plants used as the control in parallel to extracts from flowering plants; and there were no other plants except cocklebur used as test objects. At the same time there is a promising note also—the methanol extract data were corroborated at another laboratory (22).

Scientists' attention is attracted by inhibitors of flowering again. The concept, as before, is based on the fact that plant leaves exposed to unfavorable day-length conditions form substances which inhibit flowering (67, 169, 171, 186). However, the inhibition of flowering under the influence of metabolites formed in leaves under unfavorable day-length condition cannot prove per se that these metabolites are inhibitors of flowering. In this respect it is interesting to remember studies carried out by Bonner, Heftmann & Zeevaart (8) who succeeded in inhibiting cocklebur and *Ipomea* flowering with the inhibitors of steroid biosynthesis. They came to the conclusion that sterols are substances necessary for flowering. However, since the authors failed to demonstrate reversibility of the inhibition with the help of sterol preparations and mevalonic acid and to obtain conforming analytical data on the difference of sterol content in vegetative and flowering plants, one suspects that these inhibitors retard not only the synthesis of sterols but also of other processes as well. It means that the idea of specific inhibitors of flowering cannot be considered as experimentally proved.

We found traces of anthesins in radioactive carbon experiments on the translocation of assimilates from leaves exposed to long-day and short-day conditions to *Perilla*'s shoots. It was found that regularities of flowering are related to the rate of translocation of leaf metabolites provoking flowering under short-day conditions and leaf metabolites inhibiting flowering under long-day conditions (45).

All available indirect data, though convincing us of the existence of anthesins, cannot prove it, the problem of their isolation from plants and identification being one of the most essential problems of the physiology of plant development. It is possible that the discovery of anthesins is a matter for the near future.

In any case we can assume that gibberellins and anthesins together form the fourth interrelated system of metabolism which precedes flowering. In contrast to the three previously mentioned interrelated systems (carbohydrates—nitrogenous compounds, metal-containing oxidases—residual respiration oxidases, auxins—metabolites of nucleic acid metabolism), this system is of the greatest importance and decisively influences plant flowering.

Theories of Plant Flowering

All present theories and concepts of flowering are theories and concepts of photoperiodism since flowering or its absence are criteria of photoperiodism. Among the numerous theoretical interpretations of photoperiodism and plant flowering which have been proposed, the following are the most widespread and interesting: theory of endogenous rhythms and phytochrome theory; and the less popular hypothesis of the ratio of rates of light and dark reactions and the recently offered conception of two-phase flowering. All these concepts are based on full recognition of the role of trophic and hormonal factors, and this wider approach to the interpretation of flowering processes distinguishes them from previous one-sided theories and is a great contribution to plant development science. The difference between the mentioned concepts lies in which links in the chain of complex and many-sided processes of the interrelation of plant and environment and of the interrelation of organs and metabolism are to be considered the main mechanisms which start flowering.

Theory of endogenous rhythms.—The study of various aspects of life in daily and other cycles corroborated the correct suggestion that plants possess endogenous rhythmicity. Bünning's experiments (13, 14) were the earliest in this direction; they showed the transposition of phases of daily periodicity of leaf motions in long-day and short-day species: in the former leaves are erect in the evening and in the latter, in the morning. Based on this fact a hypothesis was first put forward about the connection of the photoperiodic reaction of flowering with the endogenous daily rhythm which has two phases in all plants: a photophilous phase, which occurs in the light and is characterized by high synthetic ability, intensive photosynthesis, and weak respiration; and a skotophilous phase, which occurs in the darkness and is characterized by an increase in the hydrolytical ability, intensive respiration, and the decomposition of starch and sugars.

Currently Bünning puts forward a conjecture (14) that daily endogenous rhythms consist of two half-cycles: the first, an approximately 12 hour period, coincides with the light period and is characterized by the dominance of synthetic processes; the second half-cycle is characterized by the activation of decomposition processes. Plants of both photoperiodic groups are distinguished for higher sensitivity. In long-day species one reaction from close to the skotophilous phase, takes place in darkness and in light; the second reaction, close to photophilous, depends on light and in this respect is connected with the oscillator, i.e., with the supposed intracellular biological time-recording mechanism. In short-day species the reaction, close to the photophilous phase, is the reaction which takes place in darkness and in light; the second reaction close to the skotophilous depends on darkness and is connected with the oscillator.

Bünning's theory of endogenous rhythms, as a foundation for the photoperiodic reaction of flowering, is of phenomenological nature for it is based on admission of the existence of the hypothetical central oscillator (autofluctative process) and is not easily explained from the point of view of metabolic transformations. Moreover, presently there are data that plant flowering can proceed at the same rate under different proportions of light and darkness not only in 24-hour but also in 13- and 16-hour cycles (135). At the same time it is quite admissable and useful to study the photoperiodic reaction of flowering considering historically formed and hereditarily fixed endogenous rhythms and the internal coordination of physiological processes.

Phytochrome theory.—The discovery of phytochrome came as a result of the study of the action spectrum of the visible light effect on morphogenesis, especially on plant flowering; it was carried out by Borthwick, Parker, Hendricks and others and showed that in short-day species of cocklebur soybean, *Amaranthus,* and *Chrysanthemum* red light given in the middle of the dark period prevented plant flowering, but after the subsequent treatment with far-red light these plants started flowering; in long-day species —barley and henbane—red light, on the contrary, stimulated flowering and the subsequent effect of far-red light stopped it. This phenomenon was used as an argument that there is a special pigment-phytochrome with two interconvertible forms (9, 25, 65).

It is supposed that the P_{730} form of phytochrome, which suppresses flowering of short-day species and hastens flowering of long-day species, is physiologically active. The duration of the transformation of P_{730} into P_{660} in darkness is equal to the critical length of night; therefore the formation of metabolites necessary for flowering and for flowering hormones does not take place until the transformation of P_{730} to P_{660} is accomplished, i.e., until the accomplishment of its transformation into a form able to absorb red rays. Under long-day conditions phytochrome retains the P_{730} form for longer time, and this stimulates flowering of long-day species and oppresses flowering of short-day species. Under short-day conditions phytochrome retains the P_{660} form for longer time, and this stimulates flowering of short-day species and inhibits flowering of long-day species.

The isolation of pure phytochrome from seedlings of different plants grown in darkness was a great achievement (18, 174). It was found that phytochrome is a substance whose chromophoric group is closely linked with protein; phytochrome differs from chlorophyll in its absorption spectrum, especially in the relatively weak absorption of blue rays.

The effect of phytochrome on plants is exerted through an effect on metabolism, respiration and oxidative phosphorylation, synthesis of anthocyanins and chlorophyll, activity of enzymes as well as through the content of growth-promoting substances (10). The many-sided effect of phytochrome on plants proves that it is connected with some general metabolic link or links and is not a specific system which regulates photoperiodic reactions. Phytochrome is probably one of the pigment systems which participate in the perception of the photoperiodic stimulus and in subsequent transformations of primary metabolites in plants, and this explains its importance for plant flowering.

Hypothesis on the relation of rates of light and dark reactions.—An hypothesis on the role of the activity of physiological processes during the period of light and dark reactions was first formulated by Lubimenko & Scheglova thirty years ago (118). Their experiments showed that the respiration energy in long-day species is considerable as compared with that of photosynthesis, this ratio in short-day species being much lower. In other words, the rate of dark reactions in long-day species is relatively higher and the rate of light reactions is relatively lower than in short-day species. The comparison of the nature of light and dark photoperiodic reactions, as affected by temperature and aeration conditions, also led much later to the conclusion that light reactions result in the full decomposition of specific products of photosynthesis in short-day species and their slight decomposition in long-day species, while dark reactions, on the contrary, result in the full decomposition of products of photosynthesis in long-day species and slight decomposition in short-day species, i.e., light reactions pass more quickly in short-day species and dark reactions pass more quickly in long-day species (32).

Presently this hypothesis is supplemented with additional data on the effect of temperature and inhibitors of oxidative metabolism during the treatment of plants with light and darkness. Under long-day conditions low temperature in the light inhibits or suppresses flowering of long-day species and hastens flowering of short-day species while the decrease of temperature in darkness has no effect. Under short-day conditions, on the contrary, the decrease or considerable increase of temperature in light has little effect and in darkness suppresses flowering in short-day species and stimulates it in long-day species. The same results are produced by anaerobiosis, and respiration inhibitors—cyanide, sodium azide, and dinitrophenol. When oxidative processes are suppressed during the long-day light period, there is an inhibition of flowering of long-day species and a stimulation of flowering of short-day species; when oxidative processes are suppressed in the short-day dark period, on the contrary, there is an inhibition of flowering of short-day species and a stimulation of flowering of long-day species. The effects of anaerobiosis and respiration inhibitors are barely observed, if at all, in darkness under long-day conditions and in light under short-day conditions (98).

It is necessary to remember that the nature of light and dark reactions is gradually being established. They are connected with such principal physiological processes as photosynthesis and respiration so we may hope that basic data on the nature of individual reactions and on the transformation of primary products of photoperiodism into metabolites necessary for plant flowering will be obtained through photoperiodic studies.

Conception of two-phase flowering.—The conception of two-phase flowering is based on available data on trophic and hormonal factors (41). In summing up all cited data it is possible to draw the following general picture of the role of trophic and hormonal factors of plant flowering.

Long-day species flower under long-day conditions; flowering is hastened provided that the carbohydrate content increases and the content of nitrogenous compounds is relatively low; flowering is stimulated by those segments of the respiratory process which are activated by light and which are connected with the activity of enzymes containing heavy metals; at the same time flowering is hastened provided that the auxin content of stem buds increases; a decisive effect is exerted by an increased gibberellin content in leaves.

Flowering of short-day species, on the contrary, takes place under short-day conditions and proceeds more rapidly with increase of nitrogen compounds and decrease of carbohydrates; flowering is stimulated by those parts of the respiratory process which are activated in darkness and are connected with intensification of the activity of enzymes of residual respiration; at the same time flowering is inhibited by increase of the auxin content in stem apices and is stimulated on increase of the content of metabolites of nucleic acid metabolism; a decisive role in flowering of short-day species belongs to substances of the anthesin type formed in leaves (see Table I).

TABLE I

THE EFFECT OF THE DAY LENGTH ON PLANT METABOLISM

Long day	Short day
More intensive metabolism and the increased content of	
Carbohydrates in leaves Metal-containing oxidases in leaves Auxins in stem apices Gibberellins in leaves	Nitrogenous compounds in leaves Residual respiration oxidases in leaves Metabolites of nucleic metabolism in stem apices Anthesins in leaves

The picture as printed shows that there is a close connection between nutrients and physiologically active compounds because they are associated in general metabolic processes. At the same time it contains an obvious contradiction: on the one hand, changes in the content of nutrients and hormonal substances occur in all plants under the influence of the day length in the same direction; on the other hand, some plants flower under long-day conditions and other plants flower under short-day conditions. From this it is inferred that flowering of long-day and short-day species is preceded by an opposite metabolism which seems to be quite improbable from the biological point of view.

This contradiction can be solved if our approach is evolutionary, if we admit that photoperiodic reactions are adaptive and that this helps plants to survive unfavorable environmental conditions. The genesis of long-day species from neutral followed paths such that they lost their ability to form stems during autumn short days hence to be able to survive the winter during the tillering stage under snow. The genesis of short-day species from neutral was connected with loss of the ability to form flowers during the long days of spring, hence to be able to survive the subsequent period of tropical rains or lasting droughts during the most stable stage of vegetative growth (38, 42).

Thus nature itself divided plant flowering into flower stem formation and flower formation, the realization of these developmental phases in various ecotypes depending on day-length conditions: flower stem formation in long-day species requires only long-day conditions; flower formation in short-day species requires only short-day conditions.

All this enables us to assume (39) that flowering of all annual seed plant species passes through two phases: (*a*) flower stem-formation phase; and (*b*) flower formation phase. The first phase requires the intensification of carbohydrate metabolism and respiration through metal-containing enzymes, and increase of the gibberellin content in leaves, and of auxins in stem buds. The second phase requires the intensification of nitrogenous metabolism and residual respiration, increased content of substances of the anthesin type in leaves and of metabolites of nucleic acid metabolism in stem buds (Fig. 2).

The existence of anthesins as hormonal factors of flowering has not been proved, and this is the weak point of the whole concept. It was achieved neither in experiments on the effect of extracts in bioassays on short-day species under long-day conditions—nor in grafting experiments, though we have seen that there were attempts to approach the solution of these problems.

In this connection and in results based on experimental graftings of a long-short-day species *Bryophyllum daigremontianum* where vegetating scions, being grafted to differently induced root-stocks, flowered under long-day conditions, Lang (109, 205) came to the conclusion that gibberellins are in fact florigen's precursors.

Conception of two-phase flowering allows us to approach the solution of photoperiodism and vernalization riddles.

THE MYSTERY OF PHOTOPERIODISM

The discovery of photoperiodism played a prominent role in the theory of plant development, and at the same time, it added one more mystery connected with the essence of photoperiodism, namely the entirely opposite reaction of long-day and short-day species to day length.

The outlined conception of two phases in plant flowering allows us to approach the solution of this problem. It is known that plant flowering is the main criterion of photoperiodic reaction, but this criterion is not the same when applied to two groups of long-day and short-day species originating from the common group of photoperiodically neutral species. The formation of flower stems is the critical phase of flowering in long-day species; it occurs only under long-day conditions; the ability to pass the second phase is as steady and fixed in them as it is in neutral species. The second phase

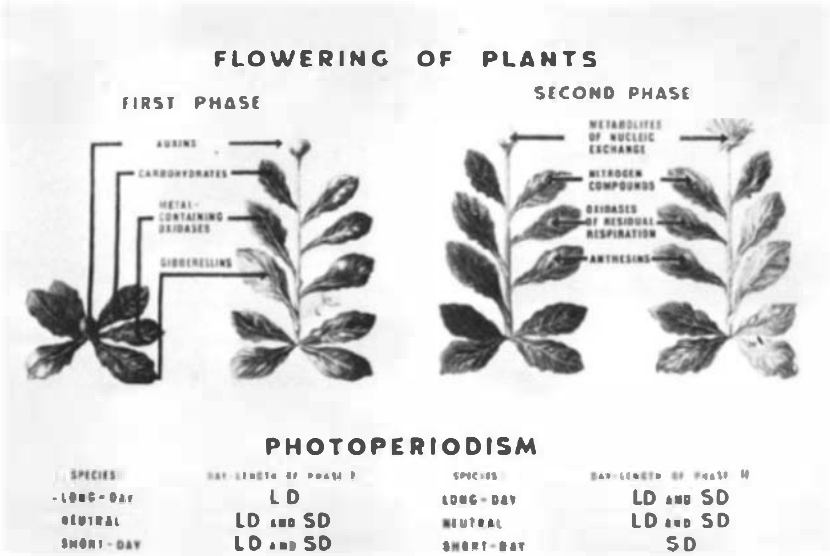
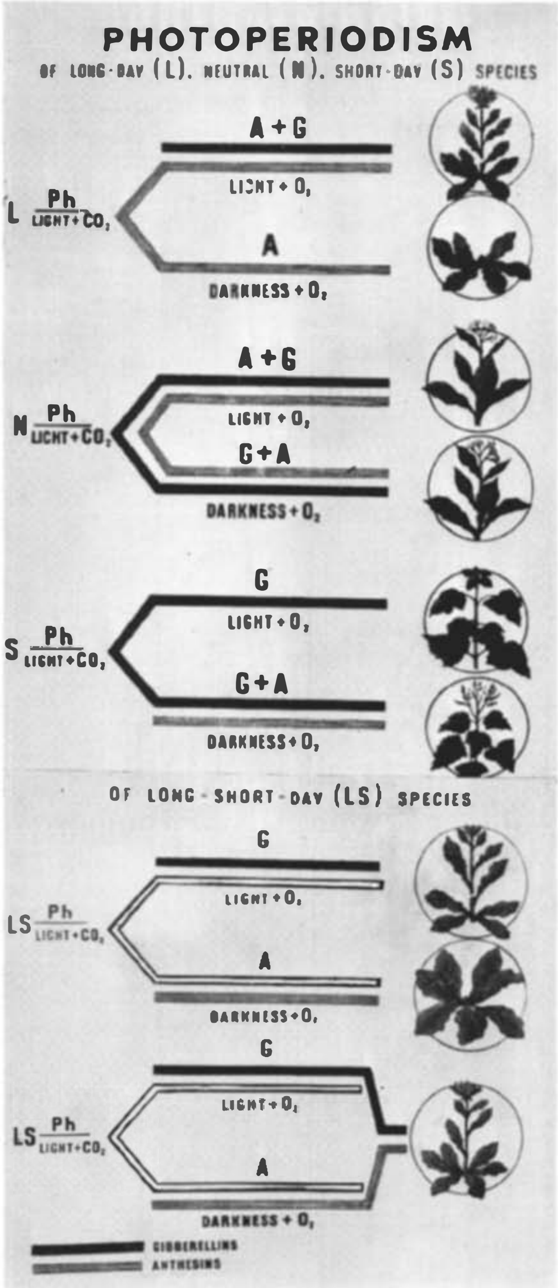


FIG. 2. Flowering and photoperiodism of plants. The first flowering phase, formation and growth of stems, is characterized by the intensification of metabolism of auxins in stem buds and of carbohydrates, gibberellins, and metal-containing oxidases in leaves. The second flowering phase, flower formation, is characterized by the intensification of nucleic acid metabolites in stem species and of nitrogenous compounds, anthesins, and residual-respiratory oxidases in leaves. Long-day species pass the first stage under long-day (LD) conditions; neutral and short-day species pass the first stage under long-day and short-day (SD) conditions. Long-day and neutral species pass the second phase under long-day and short-day conditions; short-day species pass the second phase under short-day conditions.

of flowering—flower formation—is critical in short-day species; it occurs only under short-day conditions; the ability to pass the first phase is as steady and as fixed in them as it is in neutral species (Fig. 2).

If the existence of critical phases of flowering—of the first phase in long-day species and the second phase in short-day plants—is true from the point of view of adaptation laws there remains the question of proving that the noncritical phases of flowering—the second phase in long-day species and the first phase in short-day species—can occur under unfavorable day-length conditions.

The more general answer to this question is given by studies which show that provided that there are sufficient stores of nutrients and physiologically active compounds, many plants start flowering in permanent darkness despite the nature of their photoperiodic reaction. This observation proves that all plant species possess a potential ability for flowering which displays itself under favorable day-length conditions and is inhibited or fully stopped under unfavorable day-length conditions. A more substantial answer with


PHOTOPERIODISM
OF LONG-DAY (L), NEUTRAL (N), SHORT-DAY (S) SPECIES
A + G
LIGHT + O₂
L Ph / LIGHT + CO₂
A
DARKNESS + O₂
A + G
LIGHT + O₂
N Ph / LIGHT + CO₂
G + A
DARKNESS + O₂
G
LIGHT + O₂
S Ph / LIGHT + CO₂
G + A
DARKNESS + O₂
OF LONG-SHORT-DAY (LS) SPECIES
G
LIGHT + O₂
LS Ph / LIGHT + CO₂
A
DARKNESS + O₂
G
LIGHT + O₂
LS Ph / LIGHT + CO₂
A
DARKNESS + O₂
GIBBERELLINS
ANTHESINS

regard to long-day species is given by experiments in which rosette plants of long-day species started flowering under short-day conditions under the effect of gibberellin or relatively high temperatures. An answer with regard to short-day species comes from the facts that all short-day species form flower stems both under long-day and short-day conditions; besides some short-day plants, for example, Maryland Mammoth, flower at moderate temperatures under long-day conditions.

The study of the dynamics of changes of natural gibberellins showed that gibberellin content in leaves of big tobacco plants consistently falls in permanent darkness and increases in light (48).

Having no direct experimental data on substances of the anthesin type, it may be supposed that they are formed in darkness under short-day conditions but that their formation is suppressed at light under long-day conditions. Photoperiodic reactions of long-day, short-day, day-neutral, and long-short-day species under long-day and short-day conditions are carried out in conformity to these changes of the dynamics of gibberellin content and substances of the anthesin type (Fig. 3).

Our suggestions and schemes of photoperiodism for species belonging to four principal photoperiodic groups do not claim to be considered accomplished and universal; they are of provisional nature and must be corroborated experimentally, especially for dynamics of changes of substances of the anthesin type.

It must also be pointed out that the study of factors of plant flowering is closely related with the physiology of heredity because in the very phenomenon of photoperiodism and flowering we find differently combined properties which are either variable and easily controlled by environment or are steady and fixed.

The Mystery of Vernalization

The discovery of the thermoinduction phenomenon first in winter forms and then in seedlings of biennial plants was mystery to scientists: what inhibits the generative development and flowering of these forms in the absence of lower vernalizing temperatures, and what metabolic products, formed under the effect of these temperatures, enable them to react to day-

FIG. 3. Photoperiodism of different photoperiodic groups in connection with the formation of endogenous gibberellins and anthesins. The first period of photoperiodism is the same for all four photoperiodic groups—in long-day (L), neutral (N), short-day (S) and long-short-day (LS) species. During the second period, long-day species with their stable ability to form anthesins flower only under long-day conditions when gibberellins are formed; short-day species with their stable ability to form gibberellins flower only under short-day conditions when anthesins are formed; neutral species are able to form both gibberellins and anthesins under any day-length conditions. Long-short-day species have no such stable ability; they flower under the successive alternation of long-day and short-day conditions with successive formation of gibberellins and anthesins.

length changes and to pass to flowering? In spite of the fact that this riddle is as old [1918; Gassner (70)] as the riddle of photoperiodism [1920; Garner and Allard (69)]; in spite of the fact that there are numerous experimental data and important findings (109, 139, 153), we are far from its solution and can only build up conjectures.

Some time ago, based on experimental graftings of vernalized henbane plants to annual rosette seedlings of biennial henbane, Melchers (129) came to the conclusion that vernalization results in the formation of specific substances of the hormonal nature-vernalins which are present in all spring forms. In long-day species they are directly transformed into flowering hormones under long-day conditions—in short-day species under short-day conditions. Recently, analyzing this problem, Lang (109) has also come to think that there is a direct connection between vernalin and florigen, i.e., the complex of flowering hormones; his vernalization scheme was as follows: low temperature → thermoinduced conditions → vernalin → florigen.

Sarkar (168) carried out a special study and found no correlation between the effect of vernalizing lower temperatures and the effect of gibberellic acid on plants of the winter form of *Arabidopsis thaliana;* he came to the conclusion that vernalin is not identical to gibberellin. Such difference in the effect of gibberellins and vernalization was observed by many authors (54, 106, 132, 152).

The comparative study of the content of natural gibberellins in spring and winter forms showed that leaves of spring forms of wheat, rape, and rye contained more gibberellins than corresponding winter forms (51). At the same time it was found that the content of natural gibberellins in leaves of winter forms of wheat, rape, and rye which had been vernalized, i.e., exposed to the thermoinduction at lower temperatures increases, their level almost reaching the level of gibberellins in spring forms (51, 91). The same was observed in biennial henbane, *Althaea* and *Chrysanthemum* (76, 111).

One might think that gibberellins are formed and accumulated during vernalization of plants. In fact the content of natural gibberellins in spring, winter, and vernalized forms of wheat and rape under short-day conditions is the same, and in rye it is almost the same (51, 91). Thus there is no formation and accumulation of gibberellins during vernalization; there is only a preparatory process which results in the formation of metabolites which can be considered as gibberellins' precursors (39). As a matter of fact these gibberellins' precursors are vernalins which later, under long-day conditions, are transformed into gibberellins while under short-day conditions this transformation does not take place. All spring forms produce vernalins without vernalization. It is quite probable that diffusates from seeds and sap from vernalized material of radish and winter rye plants, with which Tomita (188) hastened flowering in radish and the formation of stems in winter unvernalized wheat, contained vernalins.

There is no direct connection between vernalins and florigen because under long-day conditions vernalins turn into gibberellins which form one of

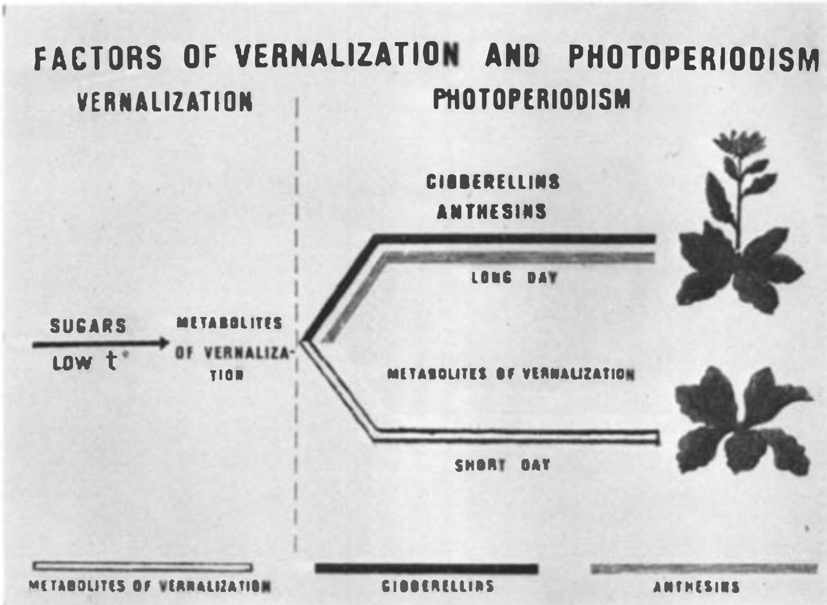


FIG. 4. Factors of vernalization and photoperiodism of plants. Winter plants form metabolites of vernalization (vernalins) as a result of oxidizing—reducing transformations of sugars at low positive temperature. Under long-day conditions these metabolites of vernalization (vernalins) turn into gibberellins, under short-day conditions such transformation is not observed. Winter dicotyledons, which are long-day species too, flower under long-day conditions and do not flower under short-day conditions.

two florigen groups (complex of flowering hormones). It can be supposed that the other group—anthesins—in winter vernalized plants is formed as in spring forms because species which include spring and winter forms and biennials are long-day species. However, seedlings of winter forms (except grasses) and biennial forms, being treated with gibberellins, form flowers only under long-day conditions and stay at the stem formation stage under short-day conditions (33, 106); this testifies to the fact that anthesins are produced in these forms only under long-day conditions (Fig. 4).

Thus with regard to internal factors of flowering, seedlings of winter and biennial, vernalized forms are not identical to those of spring forms. The critical phase of flowering in spring long-day species is marked with stem formation and preceding formation of gibberellins; the ability to form anthesins is steadily fixed in them. In winter forms and biennials both the formation of gibberellins and the formation of anthesins are critical; they pass successively, in the process of vernalization (formation of vernalins) and photoperiodic induction with long-day conditions (formation of gibberellins and anthesins). It is probable that the simultaneous adaptation of winter forms and biennials to lower temperatures and long-day conditions was connected with the loss of their fixed ability to form gibberellins and anthesins. From this point of view the more complex nature of the ontogenesis of winter plants as compared with the ontogenesis of spring forms is clear.

Conclusion

Flowering is an important stage in a plant's life cycle because the transition from vegetative growth to generative development involves essential changes in metabolism and translocation of nutrients and arouses essential formative processes which are connected with the development of organs of sexual reproduction and alteration of generations. In this field it is especially urgent to study the whole system of interrelations which exist in the plant as a whole and consequently to use widely methods of the differential application of physical and chemical factors, surgical methods, isotope methods, methods of the extraction of endogenous substances from plants and methods of biological tests and reactions. One must also make wide use of summarizing constructions and schemes which, being only physiological and not exact chemical representations, all the same help to sum up results of numerous studies and give the general characteristics of the principal regularities of plant flowering. At the same time many phenomena of plant flowering can be studied with the help of accurate and elaborate methods and gradually become translated into the precise language of physics and chemistry. There is a trend to study complex reactions which take place in cells and subcellular structures, connected with deepest aspects of their functions. This broad approach to research methods in order to study all—from the physiology of cells and subcellular structures to the physiology of the whole plant—is a reliable basis for the further understanding of all life processes, including flowering of plants.

The discovery of the role of trophic and hormonal factors is not enough to draw the overall picture of such many-sided processes as flowering and interrelated photoperiodic and vernalization processes.

The same effect obtained as a result of the photoperiodic induction with long-day conditions and chemical induction with gibberellins or the same effect of vernalization with lower temperatures and the chemical vernalization with gibberellin does not mean that chemical induction and chemical vernalization fully reproduce these processes but only testifies to the fact that we have found some principal expression of those metabolic transformations which result in plant flowering under environmental conditions where they have not been observed before. At this point scientists face an important task—to connect studies of the physiology of flowering with structural transformation, especially with the transformation of apices in which new tissues and organs are born. It should be stressed that the discovery of the most intimate relations which develop between metabolic changes and the earliest transformations in meristems of apices, which result in the development of leaf and flower rudiments, is one of the most important problems of present-day biology.

The further study of flowering processes inevitably brings us to problems of a genetic nature. The evolution of all known photoperiodic groups and their adaptation to environmental conditions is connected with changes in the hereditary apparatus of developing species; some properties remain tightly fixed; others fall beyond the direct control of hereditary information, and are controlled by environmental factors. And if physiology of plants, especially physiology of flowering, for many years did not want to deal with problems of physiology of heredity and genetics, now this task becomes the most urgent for the proper understanding of the numerous phenomena of plant life, including flowering processes.

Flowering is a decisive stage in plant life; it attracts scientists' attention because it precedes fruiting connected with the yielding ability of crops. The united efforts of scientists of many countries were required to solve the problem of yields to arrive at those great achievements which were made during recent decades; they helped to solve many mysteries of plant life and gave man efficient methods for the control of plant growth and development.

The old truth is again true—there is nothing so practical as a thoroughly elaborated theory.

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