LIGHT AND LEAF GROWTH IN PHASEOLUS

by

Diane Murray

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SUMMARY

This investigation was designed to study the role of light in the development of the primary leaves of the French bean (*Phaseolus vulgaris* (L) Savi.) The preliminary investigation studied the development of the primary leaves grown either in complete darkness, or under normal light conditions of twelve-hour days. It was found that light regulates both cell division and cell expansion; cell number and cell volume are much lower in the dark-grown leaves.

The next series of experiments on primary leaves studied the effects, on cell number and volume, of exposing dark-grown plants to various periods of illumination, with light of various wavelengths. The results indicated that light-induced cell division and cell expansion are controlled through two different mechanisms, since cell division in the leaves is stimulated by a brief (1 minute) exposure to light, while, for a marked increase in cell volume to occur, illumination of the leaves for six hours or more is necessary. It was found that red light was active in the stimulation of cell division by brief illumination, and, furthermore, that the phytochrome system was involved, since the effect of the red light can be nullified by subsequent irradiation with far-red light. Brief illumination with blue or far-red light had no effect on cell division in the leaves.
Red, blue and far-red light were all found to be equally effective in stimulating cell expansion when the leaves were exposed to prolonged illumination. It was also observed that this treatment had an effect on the cell division mechanism and resulted in a final cell number greater than that found when plants were exposed to brief illumination.

Further investigation of the phytochrome-mediated stimulation of cell division has shown that the light stimulus can be perceived either by the leaves or by the cotyledons, suggesting some involvement of the cotyledons in the response. Further results indicate that there may be some direct involvement of the cotyledons in the light response other than the supply of 'building blocks' to the growing leaves.

Biochemical changes in the leaves resulting from brief illumination have also been studied. No chlorophyll synthesis is observed indicating that photosynthesis is not involved in the response. Ribonucleic acid and protein content of the leaves increases as a result of brief irradiation, but there is no way of telling whether this is a primary or a secondary effect of the light treatment. Changes in the deoxyribonucleic acid content of the leaves have also been studied.

It would appear that light controls the development of the primary leaves through two mechanisms which differ at least in part. Stimulation of cell division through the phytochrome system is the better defined of the two, though, as yet,
the method of action of phytochrome is not known. The
role of light, when prolonged periods of illumination are
involved, is more complex, since other factors, such as the
development of the photosynthetic mechanism, must be taken
into account.
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A General

The controlling effects of light on leaf development were discovered by Ray as early as 1686, and later this phenomenon was investigated by Bonnet (1754) who observed that in darkness, beans, peas and vine branches produced small, yellow leaves. A detailed study of the development of ninety-seven different species in light and in darkness was made by MacDougal and described in his monograph produced in 1903. He observed great variation in the role of light in the development of leaves and showed that when plants were grown in darkness, leaf development varied from that shown by Narcissus sp., where the leaves were bigger in darkness than in light, and Calla sp., where the dark-grown leaves differed from those grown in light only in the absence of chlorophyll, to that shown by Vicia which exhibits what is considered the 'normal' etiolation characteristic of reduced leaf development in the dark. This has been discussed by Williams (1956) who suggests that the variation in the dark development of the leaves of the different species reflects the degree to which the leaf is composed of true mesophyll or of tissue derived from vein or petiolar material.

'Normal' etiolated characteristics are demonstrated by plants of the French bean (Phaseolus vulgaris) which when grown in darkness exhibit the following features: a greatly elongated
hypocotyl; greatly reduced development of the primary leaves, reduced development of the leaves and internodes above the primary leaves and little development of the floral primordia. In the present investigation, the growth of the primary leaves of Phaseolus was studied with a view to obtaining some information about the role of light in leaf development.

Investigations into the effect of light on leaf development were reported by Priestley (1925) and by Trumpf (1924a, 1924b.). Priestley described the effect on the development of dark-grown seedlings of Vicia and Pisum of exposing the seedlings daily to brief periods of illumination with white light. He observed that daily illumination for periods of two minutes had a marked effect on seedling development, and observed opening of the plumular hook and unfolding of the leaves as a result of these brief treatments. Longer daily exposures to light produced a greater effect on leaf development. Priestley observed that short light-treatments could produce morphological changes in the plants in the absence of chlorophyll, although he did not actually measure chlorophyll content. He considered that the morphogenic effect of light did not involve photosynthesis as had been thought previously. Priestley also indicated the importance of ensuring that plants were kept in complete darkness during the growing period. However, in handling his plants he used a ruby safelight.

Trumpf, in experiments using the Scarlet runner bean (Phaseolus multiflorus) observed that irradiation of dark-grown
seedlings with white light for 10 minutes, 30 minutes, or 2 hours daily, resulted in larger laminae and shorter internodes than were observed for the dark-grown control plants. It was claimed that chlorophyll synthesis did not occur as a result of any of these treatments. Trumpf also compared the effect of blue and red light on dark-grown plants. Using double-walled bell jars filled with lithium carmine solution to transmit red light, and ammonia and copper sulphate solution to transmit blue, he observed that while red light produced lamina expansion, blue light had no effect on the plants. Trumpf also showed that illumination of dark-grown plants kept at 3 - 4°C, had a morphogenetic effect on the plant when it was returned to darkness at a higher temperature. These experiments on the effects of light qualities are interesting in view of more recent work. This and other early work has been reviewed by Burkholder. (1936)

More recently, work investigating the effect of white light on the development of dark-grown leaves has been reported by Parkinson (1950) and Butler (1963). Parkinson, working with peas, showed that the cell-division mechanism of pea leaves was very sensitive to light. Daily illuminations with white light for one second served to increase the cell number of dark-grown leaves. Parkinson also claimed that brief illuminations resulted in increases in cell volume; however his method of estimating changes in volume of the cells, which was based on differences in area and cell number ratios of dark-and light-grown leaves, was possibly not very accurate. Butler, using the broad
bean, *Vicia faba*, showed that light could stimulate both cell division and cell expansion in dark-grown leaves, but that the sensitivity to light of the two processes differed. He observed that for plants grown in continuous light at low intensities, (0.1 - 10 f.c.) the increase in leaflet area was due mainly to cell divisions, mean cell volume remaining constant. When higher intensities of light were used (100 - 1000 f.c.) increased cell volume of the leaflets resulted. Butler went on to suggest that perhaps two different systems existed in leaves, each sensitive to different qualities of radiation and controlling either cell division or cell expansion.

Investigation of the effects of different wavelengths of light on the growth and development of plants, led to the discovery of a light-sensitive pigment system which has been shown to be morphogenically active and to be involved in the etiolation phenomenon. This light-sensitive pigment system was first discovered by Flint and McAlister in 1935. They observed that red light at a wavelength of 660 nm promoted lettuce seed germination, while far-red light (720 nm) inhibited this red-induced germination. Borthwick, Hendricks, Parker, Toole and Toole (1953) studied the effect in more detail. They determined the action spectrum for promotion and inhibition of germination, and showed that if the seeds were exposed to a sequence of alternating red and far-red radiations, the last radiation of the sequence determined the germination response. It was proposed that the following pigment system is active in the reaction:
a red-absorbing pigment (absorption maximum at 650 - 660 nm) which is inactive in the seed and which on illumination with red light (600 - 700 nm) is converted to a far-red-absorbing form (absorption maximum at 720 - 730 nm) which is active in promoting germination. The far-red-absorbing form of the pigment, on irradiation with far-red light (700 - 750 nm) can be re-converted to the red-absorbing form thus preventing germination of the seed. This pigment has been called the phytochrome system.

The phytochrome system has been found to be widespread in green or potentially green plants. However, before considering its physiological involvement in the plant, its physical and chemical properties will be considered.

Phytochrome has been extracted and studied in vitro, though as yet it has not been crystallised. It has been shown to be a blue chromoprotein, and the evidence is that the holochrome consists of one or more prosthetic groups of bilitriene, a linear tetrapyrrol, the exact structure of which has not yet been determined. The pigment exists in two forms, \( P_r \), which has absorption maxima at 665 nm and 380 nm; and \( P_{fr} \), which absorbs at 725 and 410 nm. As well as red light being active in converting \( P_r \) to \( P_{fr} \), and of far-red light in converting \( P_{fr} \) to \( P_r \), blue light at about 400 nm will drive either form of the pigment to an intermediate form. The discovery of a number of intermediates between \( P_r \) and \( P_{fr} \) (Briggs and Fork 1965) indicates that a complex system is involved in the reaction. A difference in the stability of \( P_r \) and \( P_{fr} \) has been observed,
Pr is stable in the dark while Pfr decays rapidly. Thus in the dark, following a brief illumination with red light, the Pfr formed as a result of the irradiation disappears, either being destroyed or being converted to Pr. Much of the work on the structure of phytochrome and the nature of the reaction has been done by the research group at Beltsville, Maryland, and detailed reviews of the work carried out have been made by Borthwick (1964), Siegelman and Butler (1965) and by Hillman (1967).

After the initial discovery that the phytochrome system controls lettuce seed germination, it was found that it also controls many other photomorphogenic responses. Photoinduction of flowering has been shown to be mediated by phytochrome; the effects of phytochrome on the flowering of several genera, among them Xanthium, Chenopodium, Pharbitis and Chrysanthemum, has been studied and work on this subject is discussed by Borthwick (1964).

Phytochrome has also been shown to play an important rôle in supressing the characteristics of etiolation. Downs (1955), using Phaseolus vulgaris var. Dwarf Red Kidney, showed that brief illumination of etiolated seedlings with red light (640 nm) resulted in reduced elongation of the hypocotyl and caused an increase in the length of the primary leaves. The effect of the red light could be nullified if it was followed by a brief illumination with far-red light (730 nm). These results showed that at least part of the morphogenic effect of light on plant development is mediated through the phytochrome system.
Klein, Withrow and Elstad (1965) studied the light-induced opening of the plumular hook in etiolated Phaseolus seedlings, and found that this effect was reversed by far-red light. Mohr (1964) investigated the effect of red and far-red light on etiolated mustard seedling (Sinapis alba) and listed nine characteristics of the etiolated seedling which are influenced by phytochrome, among them cotyledon expansion, anthocyanin production, differentiation of the primary leaves, and inhibition of hypocotyl lengthening.

The effect of light and the involvement of the phytochrome system in the development of etiolated tissue has also been studied using leaf discs cut from the leaves of dark-grown plants. Light effects on discs have been investigated by Liverman, Johnson and Starr (1955); Miller (1956) and by Powell and Griffith (1960), all of whom showed that discs, cut from the leaves of etiolated French bean plants, expanded on illumination with red light and that this effect could be reversed by far-red light. Both Miller, and Powell and Griffiths claimed that this increase in disc size was the result of cell division in the discs, and observed that light had little effect on cell expansion. However, in neither paper are cell number data quoted. Klein and Wansor (1963) also studied the growth of discs cut from etiolated bean leaves and observed that brief illumination with blue or red light induced disc expansion by cell multiplication. Both effects were negated by irradiation with far-red light. Actual cell number counts were made by these authors, but not published.

As well as investigations into the morphogenic changes in etiolated mater...
etiolated material mediated through the phytochrome system, biochemical changes occurring in irradiated material have been studied. Mitrakos, Klein and Price (1965) investigated changes in the carbohydrate content of etiolated corn leaves resulting from illuminating them briefly with red light. Increased utilisation of soluble sugars in the leaf was observed, and experiments in which $^{14}$C glucose was supplied to the leaf tissue showed light-stimulated incorporation of glucose into the insoluble tissue residue, which consisted presumably of cell wall components. These results are interesting in the light of work by Dale (1966) who studied the effects of light and nutrients on the growth of discs cut from light-grown French bean leaves and observed a light-dependent uptake of sucrose by the discs.

Increases in the ribonucleic acid (RNA) and protein content of the cotyledons of dark-grown Sinapis seedlings after brief illumination with red light were observed by Mohr (1966). This increase was prevented by illumination with far-red light. Mohr (1962) also quotes work by Landgraf to show that an alteration of protein metabolism may follow formation of $P_{fr}$ or the formation of one of the products of the High Energy Reaction (see below).

Other workers have reported changes in various enzyme systems after illumination with red light (Hillman and Galston 1957, Marcus 1960, and Keister, Jagendorf and San Pietro 1962) and the phytochrome system has been shown to be involved in chlorophyll synthesis (Mitrakos 1961) and (Sisler, Klein and Gettens 1961).
The number and variety of the responses regulated by phytochrome makes it difficult to determine whether a given response is a primary result of the light treatment, or whether it is brought about indirectly as a result of an effect of light on a separate reaction system.

So far, only the morphogenic effects of short light-treatments have been considered. Prolonged illumination has considerable effects on plant growth and development, and these are now considered. Work on the formation of anthocyanin in the cotyledons of Sinapis alba has been extensively studied by Mohr and co-workers (Mohr 1957, Mohr 1962, Wagner and Mohr 1966) who have shown that it can be controlled through a mechanism including the phytochrome system. However, when the production of anthocyanin in continuous light was studied it was found that far-red and blue light were more effective than red light in promoting anthocyanin synthesis; the action spectrum for the response showed a major peak in the far-red at 730 nm and a smaller, broader peak in the blue at 400–500 nm. This response is referred to by Mohr as the High Energy Reaction of Morphogenesis (HER). He has also shown that cotyledon expansion in Sinapis and hypocotyl growth in Lactuca can be controlled through this system. Both these responses show similar action spectra as that found for anthocyanin production.

Mohr has explained the HER through the action of phytochrome. (Wagner and Mohr, 1966) He argues that because the equilibrium state of phytochrome in far-red light is $95\% P_r$ and $5\% P_f$,
continuous illumination with far-red light would result in a small constant amount of $P_{fr}$ being present in the plant throughout the period of illumination. (Any destruction of $P_{fr}$ that occurred would be followed by rapid replacement from the pool of $P_{r}$.) However, in red light, 80% of the phytochrome is present as $P_{fr}$ which is rapidly destroyed and cannot be replaced. The continued maintenance of a small quantity of $P_{fr}$ in far-red light is thought to be more effective in producing the morphological response. Continuous illumination with blue light could also act through the phytochrome system since both $P_{r}$ and $P_{fr}$ have absorption peaks in the blue region of the spectrum.

Recent work by Clarkson and Hillman (1967) has shown that repeated exposure of pea seedlings to red light resulted in an apparent synthesis of phytochrome once the endogenous level had fallen to 17% or less of the initial value as a result of light mediated destruction. No apparent synthesis of phytochrome was observed in dark grown plants. This finding, if generally confirmed, will require some extension and redefinition of Mohr's interpretation of the HER.

A further theory to account for the HER has been proposed by Evans, Hendricks and Borthwick (1965). They maintain that the HER is not entirely phytochrome mediated, and suggest that it involves the formation of materials necessary for the working of the phytochrome mechanism. They base this theory on work carried out with lettuce and Petunia hypocotyls. They observed that a brief illumination with red light caused inhibition of
hypocotyl extension only if it was preceded by a prolonged period of illumination with either blue or far-red light. This red-light-mediated inhibition could be reversed by a subsequent brief illumination with far-red radiation. These results were taken to indicate an interaction between the HER and the phytochrome system. Similar conclusions were reached by Lane and Kasperbauer (1965) who investigated the effect of various light treatments on twining of dodder seedlings. Vince and Grill (1966) working on anthocyanin synthesis in turnip seedlings also obtained results which are in accord with this interpretation.

The work which has been discussed so far shows that it is imperative that the effects of both brief and prolonged periods of illumination be studied. Since two different reaction systems may be involved, it is advisable that they be studied separately.

The mode of action of phytochrome in producing its varied physiological and morphogenic effects is unknown. It has been suggested by Mohr (1966) that phytochrome may produce its effects through activation of potentially active genes. This view is based on observations of increased RNA synthesis in Sinapis cotyledons as a result of brief illumination with red light or of prolonged illumination with far-red. Investigations using inhibitors of RNA synthesis also provide evidence that light-stimulated RNA synthesis is involved in the photomorphogenic response of the plant. However, the discovery that light can induce morphological changes before marked changes in RNA content are observed makes interpretation of the mode of action of phytochrome
more difficult (Weidner and Mohr 1967).

Hillman (1967) mentions other theories of phytochrome action which suggest either that phytochrome may be an enzyme controlling some basic process in the plant, or that it may act through altering membrane permeability. Although as yet there is no conclusive evidence as to the mode of action of phytochrome, the variety of the responses which can be controlled by this system indicate that it must act through some basic cellular mechanism. Work on the action of phytochrome and of the High Energy Reaction System has been reviewed by Wassink and Stolwijk (1956), Mohr (1962, 1964) and Hillman (1967).

B The Nature of the Investigation

In the present investigation, the development of the primary leaves of Phaseolus in darkness and in light has been examined in detail. Plants of Phaseolus vulgaris were chosen as the experimental material for the following reasons:—

1. Phaseolus exhibits very marked etiolation characteristics. There is a very great morphological difference between dark-grown and light-grown plants.

2. Phaseolus has a large seed and carries ample food supplies in the cotyledons. This is an advantage in the investigation of etiolation since the seedlings can be grown for a considerable period in the dark without additional organic food supply.

3. When grown at temperatures greater than 20°C, germination
of the seed and development of the seedling is rapid. This makes it convenient for experimental work.

4. Since growth of the primary leaves is examined in detail in this study, the presence of these primary leaves as well-formed structures in the seed facilitated handling of the material from the earliest stages of an experiment.

5. Data obtained by Dale (1964a, 1964b, 1965) on the effect of temperature on leaf growth and on the light-development of the seedlings as a whole and on the leaves in particular were useful in setting up preliminary experiments and in the initial characterisation of the normal development of the leaves.

As already discussed, experiments by earlier workers on the controlling effect of light on leaf development have shown that etiolated leaves are extremely sensitive to light and it has also been shown that the phytochrome system is involved in the response of the leaves to radiation. However, many of these investigators have observed the stimulatory action of light merely as effects on the length or fresh weight of the leaves. In the present investigation, cell division and cell expansion of the leaves has been studied and the rôle of light (acting through the red, far-red reversible phytochrome system and the High Energy Reaction system) in controlling the two processes examined. Internal biochemical changes as a result of different light treatments, and the involvement of different parts of the plant in the response of the leaves have also been studied to ascertain the importance of internal factors on the light response.
A Material

The material used for this investigation was the French bean (*Phaseolus vulgaris* (L) Savi.). Two varieties were used; cultivar Sutton's Selected Canadian Wonder and cultivar Sutton's Masterpiece. The former variety was used for most experiments, but Masterpiece was used over a short period when the stock of Canadian Wonder ran out before the new season's supply was available. Unless stated in the text, the variety used was Canadian Wonder.

Seeds within the weight range 0.4 - 0.6 g. were used. This was the range which contained most of the seeds; very large and very small seeds were discarded.

The pattern of development of the primary leaves in the dark and their reaction to various treatments was the same for Masterpiece as for Canadian Wonder, but the leaves of Masterpiece were slightly smaller. Since the cell number was similar in both varieties, the smaller size of Masterpiece leaves was due mainly to a smaller cell-size. (This difference can be seen in Fig. 1).
Fig. 1.
Changes with time in the fresh weight (a) and cell number (b) of dark-grown primary leaves of *Phaseolus* cultivars Masterpiece (open symbols) and Canadian Wonder (closed symbols).
MEO

(a).

160

Fresh weight (mg)

80

0 2 4 6 8 10 12 14 16

Age in Days.

RM

no

20

2 4 6 8 10 12 14 16

Age in Days.

240

160

60

Cell number (millions)

40

20

Canadian Wonder.

Masterpiece.
B Methods

(a) General methods

1. Culture methods

Seeds were grown in washed river-sand (Levenseat No. 13) and watered when necessary with Hoagland's mineral nutrient solution (Hoagland and Arron 1938) made up in tap water. Seeds were not soaked before planting as work by Wheeler has shown that this may result in the seedlings being smaller. (Wheeler, 1965) In all cases the age of the plants is given in days from the day of planting (Day 0).

Plants were grown in several different types and sizes of container; clay and plastic pots, clay pans and plastic seedtrays were all used. No difference was observed in the development of the plants grown in the different types of container.

2. Replication

All quoted values of area, fresh weight, dry weight, cell number or cell volume are average values for at least 5 replicate plants. The level of replication used in the determination of chlorophyll, alcohol-insoluble nitrogen, ribonucleic acid (RNA) and desoxyribonucleic acid (DNA) is stated in the appropriate part of the results section.

3. Statistics

95% confidence limits and least significant differences (LSD) (p=0.05) were calculated using standard methods. (Brownlee,
1953) The regression line shown in Fig. was calculated on the Edinburgh University KDF9 computer using an ANAS Autocode Programme drawn up by Dr. J. E. Dale.

4. Growing conditions

(i) Light conditions. Plant material referred to in the text as 'normal light-grown material' was grown in a growth room of the type described by Dale (1964a), in which temperature, light-intensity and day-length could be controlled. A cycle length of 24 hours was used (12 hours light, 12 hours dark), with a light-intensity of 800-900 foot candles. Light was generated by Warm-White fluorescent tubes and tungsten lights which gave 30 foot candles. Temperature was 22.5 ± 1°C. throughout.

(ii) Dark conditions. Two different sets of dark conditions were used. The first type (Dark cupboard) was a light-tight cupboard built across the end of a growth room such that it had the same conditions of aeration and temperature as the growth room. This was kept at a temperature of 25 ± 1°C.

It was found after some time that the wooden frame of the dark cupboard had warped, allowing light to leak in. This was discovered when a piece of unexposed film, left in the cupboard for two days was observed, on developing, to have fogged. The light which entered the cupboard was of low intensity and it was not possible to measure it quantitatively.

The leak was corrected and earlier experiments were repeated to determine its effect on the development of the primary leaves. It was found that exposure of the plants over their
Fig. 2.

Changes with time in the cell number of the primary leaves of French bean plants grown in complete darkness (squares) and when a light leak (circles) was occurring.
MOE 50
Cell number (millions).

20
Jo 4 8 12 16 20 24
Age in Days.
growing period to the low-intensity light resulting from the leak had a detectable effect on the growth of the primary leaves. Fig. 2 shows the results from several experiments demonstrating the changes with time in the cell number of the primary leaves of plants grown under absolute dark-conditions and when the light-leak was occurring. There is little difference in the curves over the first eight days, but there is a difference in the final number of cells formed. Cell divisions ceased by day 8 in the dark, somewhat earlier than under the light-leak conditions. The final cell number in complete darkness was 70% of that obtained when the light leak was occurring. Fresh weight of the primary leaves was increased as a result of the light leak, but cell volume was unaffected.

The second type of dark conditions used comprised a dark room, the temperature of which was controlled by a Rootes Tempair unit (modified to use a 1kw. heater rather than the 3kw. unit usually supplied). The room, which was entered from a dark corridor, had a heavy curtain hanging inside the door to ensure complete darkness during entry. The darkness of the conditions was tested using photographic film. The room was kept at a temperature of $25 \pm 1^\circ C$.

Except where otherwise stated, all results were obtained using this second type of dark room.

5. Handling of dark-grown material

Where it was necessary to handle dark-grown material, this
Fig. 3.
Changes with time in the fresh weight of the primary leaves of French bean plants grown under dark conditions with (open symbols), and without (closed symbols), a green safe light being used.

Fig. 4.
Changes with time in the cell number of the primary leaves of French bean plants grown under dark conditions with (open symbols), and without (closed symbols), a green safe light being used.
Fig 3.

300
200
100
Fresh weight (mg.).

Age in Days.

10 12 14

4 6 8

Fig 4.

50
40
30
20
10

Cell number (millions).

Age in Days.

10 12 14

4 6 8
was done using light from a 15-watt tungsten bulb passed through a Kodak Clear Light Green Safelight (Wratten series No. 12). This generated light between the wavelengths 500 and 560 nm and was situated 3 feet above bench level. As can be seen from Figs. 2 and 4, irradiation with this green light had no effect on either fresh weight or cell number of the primary leaves. A small handheld torch with a green filter was also used occasionally in handling material. This generated light between 440 and 580 nm and had no effect on the growth of the plants.

(b) Harvest methods

1. Dry-weight determinations

Material for estimation of dry weight was dried for 24 hours in an oven at 80°C, before weighing.

2. Measurement of leaf area

Two methods of estimation of leaf area were used.

(i) The leaf shape was traced onto paper of uniform thickness, the paper shapes were then cut out and weighed. Area was calculated by comparison with a piece of paper of known area and weight.

When small leaves were measured, the leaf was placed in a photographic enlarger and the enlarged, projected image of the leaf traced onto paper. A piece of paper of known area was similarly treated and this was used to calculate the area of the leaf.
(ii) Leaf area was also measured using leaf-area meters (Evans Electric Selenium Ltd.), which were calibrated using pieces of leaf material of known area. Light-grown material was used for calibration when calculating the area of light-grown leaves and etiolated material used for calculation of the area of dark-grown material. (Hard and Rees, 1966)

3. Estimation of cell number

Cell counts were made using a modification of the method of Brown and Rickless (1949). Material was placed in 5% chromic acid and left at room temperature over night, by which time it could be easily macerated using a 5ml. syringe with a 10cm. needle of bore 1mm. After separation of the cells, the sample was made up to a known volume and the cell number determined using a haemocytometer. Six replicate 3x3mm. fields were counted for each sample.

Material was not kept in acid at room temperature for more than two days before maceration and counting. However, it was possible to store material in a refrigerator at 4°C. for up to 5 days before counting, without any breakdown of cells.

4. Estimation of cell volume

Mean cell volume was calculated from fresh-weight and cell-number values assuming that the specific gravity and density of the material was unity.

5. Estimation of chlorophyll

The chlorophyll content of the leaves was estimated spectro-
photometrically. Leaves were extracted three times in boiling 80% ethanol, the supernatants bulked, made up to a known volume and the extract scanned using a Unicam S.P. 500 Spectrophotometer.

The main absorption peak of chlorophyll in 80% ethanol was found to be at 682nm. The optical densities (OD) of the samples were determined at this wavelength and chlorophyll content expressed as OD/leaf pair in 10ml. 50% ethanol.

6. **Estimation of Desoxyribonucleic acid (DNA)**

DNA was extracted in hot perchloric acid using the method of Nieman and Poulsen (1963) for fresh leaf material and estimated spectrophotometrically using Burton's (1956) modification of Dische's diphenylamine reaction. Calf thymus DNA was used as standard and the results expressed in µg. DNA per leaf pair or per 50 million cells.

7. **Estimation of Ribonucleic acid (RNA)**

Estimation of total RNA was made following extraction in cold perchloric acid using the method of Nieman and Poulsen (1963) for fresh leaf material. The total RNA was estimated spectrophotometrically (Unicam S.P. 500 Spectrophotometer) by determining the optical density (OD) of the samples at 260nm. OD units were converted to µg RNA assuming that an OD of 1 is given by 332 µg/ml. of RNA (Calculated by Heyes – personal communication – for RNA from rye and lupin leaves). Results were expressed in µg RNA/50 million cells or in µg RNA per leaf pair.
8. Estimation of ethanol-insoluble nitrogen

Leaf material was extracted three times in boiling 80% ethanol. The residual material was then dried for two hours in an oven at about 90°C, and the nitrogen content determined by microdiffusion analysis (Conway 1962) following sulphuric acid digestion.

A maximum of 20mg. of dried material was digested in digest acid (36N nitrogen-free sulphuric acid containing the equivalent of 0.2g./l. of CuSeO₄). The digested sample was cooled and made up to 10ml. with distilled water. Conway dishes were prepared following the method of Sunderland Heyes and Brown (1957). 0.2ml. of half strength boric acid indicator was placed in the inner well while 0.2ml. of 40% sodium or potassium hydroxide was added to one half of the outer well and 0.5ml. of the diluted extract in the other half. The glass cover was put on with a good seal using vaseline jelly, the contents of the outer well mixed, and the units left overnight.

The contents of the inner well were then titrated against N/20 H₂SO₄. The nitrogen content of the sample was calculated using the fact that 1 μl N/20 H₂SO₄ is equivalent to 0.7 μg of nitrogen.

9. Estimation of protein nitrogen

The ethanol insoluble nitrogen estimated as above consisted of protein nitrogen and also of DNA and RNA nitrogen. To determine the amount of protein nitrogen in a sample, μg RNA and DNA were converted to μg RNA and DNA nitrogen using factors
quoted by Williams and Rijven (1965). By subtracting total nucleic acid nitrogen from the ethanol insoluble nitrogen value a value for protein nitrogen was obtained.

(c) Light Treatment Methods

1. Generation of light for various treatments

The light source used when light treatments were given to dark-grown plants consisted of a rack holding three, 5 foot, 80watt, Cool-White or Warm-White fluorescent tubes and twenty, 25watt tungsten bulbs. These illuminated a shelf 20 ins. below, on which plant material was placed. The fluorescent tubes and the tungsten bulbs were wired independently and could be used separately or together. This arrangement was used for irradiation with white light and also for irradiations using red, blue or far-red light.

In some experiments, 40 watt tungsten bulbs were used for generation of far-red light. When this was done, eight of the 25 watt bulbs immediately above the material being illuminated were replaced with 40 watt bulbs. This generated a higher intensity of far-red light.

Normal growth-room light-conditions were also used in some experiments.

2. White-light treatments

Two different types of white-light treatment were given to dark-grown plants; one involved transferring plants to normal
light-conditions, generally for periods of 2 days or longer; for shorter treatments with continuous white-light the plants were illuminated on the light rack described above. Light was generated using either Cool-White or Warm-White fluorescent tubes along with the 25 watt tungsten bulbs. This gave a light intensity of approximately 700 f.c. at plant level. The type of fluorescent tube used had no effect on the response of the leaves.

In one experiment, when plants were treated with continuous light the treatment was given in a growth room; this is specifically mentioned in the text.

3. Red, blue and far-red light-treatments

(i) General Irradiation of dark-grown material with red, blue or far-red light was carried out in a specially designed box which was placed on the shelf below the light rack during illumination. The box was constructed with two, one foot square windows on the top surface. These held glass filters in grooved channels. Double grooving was used such that two different filters could be placed one above each other in each window if desired. Apart from light passing through the filters, the box was light-tight. Plants were transferred to and from the box under dark conditions or using the green safe-lights already described, thus ensuring that the only physiologically-active radiation received by the plants was that passing through the filters. In some experiments, cellophane sheets were used as filters. When this was done, the cellophane was fixed closely over the top of the box using sellotape.
Radiation was measured using a spectroradiometer (Agricultural Specialities Inc., Beltsville, Maryland) calibrated against a light source standardized at N.P.L. Values obtained are expressed as $\mu$watts/cm$^2$/nm. The values for the transmission curves of various light sources and filters which are shown in Figs. 5a and b, were obtained with the radiometer head on the floor of the box described above.

(ii) Red light. Red light was generated by filtering the light from three five foot 80 watt Cool-White fluorescent tubes through red-glass plates (Flashed ruby glass—Cunningham, Dickson and Walker, Edinburgh). The red plates transmitted no light below 516nm, while the cool-white fluorescent tubes emitted low quantities of far-red light. The use of this filter and light source produced a transmission curve as shown in Fig. 5a with a peak at 632nm with no transmission in the blue and with very low transmission in the far-red.

Red cellophane (Dennison Manufacturing Company) was used as the filter for generating red light in some cases. Cool-white fluorescent tubes were used as above, and light was transmitted over the same wavelengths as with the glass filter, with a peak at 632nm. The red cellophane, however, transmitted about four times as much red light at 655nm and twice as much far-red light at 722nm. The transmission curve is shown in Fig. 5a.

(iii) Blue light. Blue light was generated using Flashed Blue Glass filters (Cunningham, Dickson and Walker, Edinburgh). The light source was three Cool-White fluorescent tubes which, as already stated, emit a very low level of far-red radiation,
**Fig. 5. (a+b)**
Transmission curves for the various combinations of light source and filter used in the course of the investigation.

<table>
<thead>
<tr>
<th>Filter</th>
<th>Light source</th>
<th>Light generated</th>
</tr>
</thead>
<tbody>
<tr>
<td>■ Blue glass</td>
<td>Fluorescent tubes</td>
<td>Blue</td>
</tr>
<tr>
<td>○ Red glass</td>
<td>Fluorescent tubes</td>
<td>Red</td>
</tr>
<tr>
<td>▼ Red cellophane</td>
<td>Fluorescent tubes</td>
<td>Red</td>
</tr>
<tr>
<td>□ Red cellophane</td>
<td>40 watt tungsten</td>
<td>Far-red</td>
</tr>
<tr>
<td>+ Blue glass</td>
<td>40 watt tungsten</td>
<td>Far-red</td>
</tr>
<tr>
<td>△ Red cellophane +</td>
<td>25 watt tungsten</td>
<td>Far-red</td>
</tr>
<tr>
<td>▲ Red glass + Blue</td>
<td>25 watt tungsten</td>
<td>Far-red</td>
</tr>
<tr>
<td>▼ Red glass + Blue</td>
<td>25 watt tungsten</td>
<td>Far-red</td>
</tr>
<tr>
<td></td>
<td>bulbs</td>
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<tr>
<td></td>
<td>bulbs</td>
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<td>bulbs</td>
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</table>
while the blue plates transmitted very little light in the red wavelengths. The transmission curves with a peak at 440nm are shown in Fig. 5a.

(iv) Far-red light. Far-red light was generated by passing light through both red- and blue-glass plates or through red cellophane and a blue-glass filter. The light source used was 25 or 40 watt tungsten bulbs which emitted light rich in the far-red. The red-plates prevented transmission in the blue, and the blue plates cut down transmission in the red. The transmission curves for far-red light are shown in Fig. 5b. An increased intensity of far-red light was obtained by using either the red cellophane or the 40 watt bulbs or both.
III - RESULTS

Section A: The development of the shoot of Phaseolus seedlings in darkness and in light.

The object of the experiments in this section was to investigate the large differences in the development of Phaseolus seedlings in the dark and in light. The course of development of the shoot of normal and etiolated seedlings was investigated and the growth of the primary leaves was studied in detail to obtain information about the effects of light on their development. These results form a basis for more detailed studies on leaf growth.

1. Initiation and development of vegetative and floral primordia in dark or light conditions.

This experiment was designed to investigate the development of the shoot of seedlings grown under dark or light conditions. Drawings of light- and dark- grown plants are shown in Fig. 6. In etiolated plants, apart from the obvious reduction in size of the primary leaves, there is also reduced development of the leaves and internodes above the primary leaves. In this experiment, copies of light- and dark-grown plants were dissected at different ages to determine whether initiation of trifoliate leaf primordia is reduced in the dark, or whether it is the subsequent development of the leaves which is affected.

Seedlings were grown under light or dark (Dark cupboard)
Fig. 6

Six day old dark- (A) and light- (B) grown Phaseolus seedlings.
conditions over a period of twelve days. Samples containing at least six plants were harvested at different times after planting and the shoot apices dissected using fine needles. The number of vegetative and floral primordia present at each age was calculated, and diagrams were constructed to show the average number of primordia and the stage of development of the youngest primordia (Figs. 7 & 8).

a. Development in light (Fig. 7). At day 0 (i.e. in the dry seed), the shoot apex of the embryo was bare, apart from the presence of the stipular primordia of the primary leaves (Fig. 7a). Development of the apex in the light was rapid. By day 2, the first trifoliate leaf and its stipules could be distinguished and the primordia of the second trifoliate leaf and stipules were developing at the apex (Fig. 7b). By day 4, the fourth trifoliate primordium was present and by day 6, floral primordia were being produced (Figs. 7c and 7d). Initiation of floral primordia proceeded rapidly (Fig. 7e) and was complete by day 11 (Fig. 7f). After four floral primordia had been produced there was no further development of the shoot apex. These results agree with Dale (1964b) who found a similar pattern of development of the shoot apex in light-grown French bean plants.

b. Development in darkness (Fig. 8). Development of the trifoliate leaf primordia was slightly slower in darkness than in light. On average three primordia were present on day 4 in dark-grown apices (Fig. 8a) as opposed to four in the light (Fig. 7c). By day 5, the fourth trifoliate leaf had been initi-
Fig. 7

Diagrammatic representation of leaves and primordia present at the apex of light-grown Phaseolus plants of different ages.

(a) Day 0  (b) Day 2  (c) Day 4  (d) Day 6
(e) Day 8  (f) Day 11 - leaves and stipules omitted -

(See over for key to the different parts shown)
Key for Figs. 7 and 8

- cotyledons
- primary leaves
- stipules
- trifoliate leaves
- apex
- floral primordium
- developing flower
- bract enclosing inflorescence
Fig. 8
Diagrammatic representation of leaves and primordia present at the apex of dark-grown Phaseolus plants of different ages.
(a) Day 4 (b) Day 5 (c) Day 6
(d) Day 7 (e) Day 8 (f) Day 12
ated in the dark (Fig. 3b), and on day 6, the first floral primordium was observed (Fig. 3c). Although the same number of foliar primordia had been formed by day 6, they were less developed in the dark-grown plants than in those grown in the light (Figs. 7a & 8c). Development of floral primordia in the dark was much reduced and on average only two floral primordia were formed (Figs. 8e & 8f).

These results show that light during germination is not necessary for initiation foliar primordia or of floral primordia in Phaseolus. Although fewer floral primordia are produced in darkness than in light, the fact that production of floral primordia occurs at all in the dark, indicates that light is not necessary for floral induction.

Trifoliate leaf production occurred more slowly in darkness than in light, but the major difference between dark- and light-grown apices was in the subsequent development of the leaf primordia. In the light, the leaves developed very rapidly. By day 15, the primary leaves were fully expanded and the first two trifoliate leaves were expanding and unfolding from the apex. In the dark, comparatively little development of the primary and trifoliate leaves occurred. Thus light during germination is not necessary for initiation of the primordia of trifoliate leaves, but it is necessary for their subsequent development and for the development of the primary leaves.

As a basis for further studies, development of the primary leaves of Phaseolus plants grown under light and dark conditions was studied in detail. Leaf development was investigated over an eighteen day period and changes in fresh weight, dry weight, area, cell number and mean cell volume of the primary leaves were followed. Seedlings were grown either under light conditions or in continuous darkness (Dark cupboard) and the primary leaves were harvested at intervals from day 2 to day 18.

In light-grown plants, the plumular hook emerged above the sand during day 5 and unfolded on day 6. The primary leaves also unfolded on day 6 and greening due to chlorophyll formation was observed at this age. By day 7, the leaves were opened and flattened.

Dark-grown plants emerged one day earlier. Unfolding of the plumular hook in the dark was incomplete even by the end of the experiment (i.e. day 18). In darkness, the primary leaves remained folded about each other and did not flatten or unfold, nor did they green.

The earlier emergence of the plumular hook in the dark is due to faster growth of the hypocotyl in darkness than in light. The lower rate of extension of the hypocotyl in the light could be due to the inhibitory effect of light filtering through the sand in which the seedlings were growing. Light has been observed in this and other investigations to inhibit hypocotyl growth (Downs, 1955).
In light-grown plants there was an initial slow increase in the fresh weight of the primary leaves (Fig. 9). After day 6, fresh weight rose rapidly and was still increasing on day 15. In dark-grown leaves, after a slow increase in fresh weight up to day 8, a constant value was reached and there was no further significant increase.

The pattern of change in area of the primary leaves of dark- and light-grown plants was basically the same as that in fresh weight (Fig. 10). In light, the area of the primary leaves increased slowly up to day 6 and then increased rapidly. The leaves were still rapidly increasing in area by day 15. In the dark, the area of the primary leaves increased slowly until day 8 and then remained constant.

For light-grown plants, the absolute rate of increase of dry weight of the primary leaves rose up to day 11 and thereafter declined (Fig. 11). In dark-grown leaves, dry weight increased at a much lower rate, and after day 8 no further increase was observed.

The cell number of the primary leaves of light-grown plants rose rapidly until day 8 after which no further increase was observed (Fig. 12). In dark-grown leaves, cell divisions occurred over the same period, i.e. up to day 8, but the final number of cells formed was less than half the number formed in the light.

Examination of the mean cell volume of light-grown leaves showed little increase up to day 6 (Fig. 13). After day 6, large increases in cell volume occurred which were continuing on day 15. A similar pattern was followed by dark-grown leaves up to day 6,
Fig. 9

Changes with time in the fresh weight of the primary leaves of dark- (closed symbols) and light- (open symbols) grown plants.
Fresh weight (g.)
Fig. 10

Changes with time in the area of the primary leaves of dark- (closed symbols) and light- (open symbols) grown plants.
300.

200.

100.

Area (cm²).

Age in Days.
Fig. 11
Changes with time in the dry weight of the primary leaves of dark- (closed symbols) and light- (open symbols) grown plants.
Dry weight (mg)

Age in Days.
Fig. 12

Changes with time in the cell number of the primary leaves of dark- (closed symbols) and light- (open symbols) grown plants.
Fig. 13
Changes with time in the mean cell volume of the primary leaves of dark- (closed symbols) and light- (open symbols) grown plants.
Mean Cell Volume (c.c. x 10^9)

Age in Days
but the sudden increase in cell volume seen in the light did not occur.

The results for light-grown material are in agreement with those reported by Dale (1964b), who showed that the early growth of the leaves in terms of fresh weight, dry weight and area followed a logarithmic pattern. This is true also for the light-grown plants investigated here, the logarithmic phase ending at day 8 (Figs. 14a, b & c). The results also show that the increase in cell number in the leaf over the first 6 days is logarithmic in form (Fig. 14d). In darkness the increase in fresh weight, dry weight and area of the primary leaves is also logarithmic, but the rate of increase is much lower than that found in light-grown leaves. In dark-grown leaves cell number increases logarithmically over the first 8 days (Fig. 14d), this is over a longer period than in the light, but in the dark the rate of increase is much slower.

The results of this experiment can be summarised as follows: in the absence of light, growth of the primary leaves of Phaseolus is greatly reduced, the final cell number and cell volume is much lower in the dark-grown leaves, indicating that light has a stimulatory effect on the cell division and cell expansion mechanisms. The object of further experiments was to determine in more detail the nature and mode of action of this stimulus.
Logarithmic plot of the changes with time in (a) area (b) fresh weight (c) dry weight and (d) cell number of the primary leaves of dark- (closed circles) and light- (open circles) grown plants.
Section B:- The effect of various treatments with white light on the development of the primary leaves of dark-grown Phaseolus plants.

The introductory experiments showed that the greater degree of development observed in the primary leaves of light-grown plants was due to both the cell number and the mean cell volume being larger in light than in darkness. The next experiments were carried out to determine if it was possible to stimulate cell division and cell expansion in the primary leaves of dark-grown Phaseolus plants by treating them with white light. The effects of different periods of illumination were studied and the response to light treatment of leaves of different ages investigated.

1. The effect on the primary leaves of transferring plants of different ages from dark to light conditions.

   This experiment was designed to determine whether it is possible to stimulate cell division and cell expansion in dark-grown leaves of various ages by transferring them to light conditions.

   Plants grown in darkness (Dark cupboard) were transferred to light conditions 2, 4, 6, 8, 10 or 12 days after planting. Plants were harvested on the second and sixth days after transfer and the fresh weight, cell number and mean cell volume of the primary leaves were estimated. Control plants were kept in darkness throughout the experiment.

   Transfer to light conditions resulted in an increase in the
fresh weight of the primary leaves irrespective of the age of the plant at the time of transfer, but the degree of response to the light was not the same for each age of plant (Fig.15). Plants up to eight days old at the time of transfer showed a rapid increase in the fresh weight of primary leaves. The response of the older leaves on transfer to light decreased with age, and the rate of increase in fresh weight was much lower than for younger leaves.

When plants up to six days old were transferred to light conditions, there was a stimulation of cell division in the primary leaves (Fig.16) which resulted by day 8 in the cell number of the treated leaves reaching the same value as that found in normal light-grown leaves. Leaves which were 8, 10 or 12 days old at the time of transfer responded progressively less to the stimulus, so that the final cell number was less than that of the normal light-grown leaves. For material six days old or older at treatment, the effect on cell number was completed by the time of the first harvest, i.e. within 48 hrs.

When plants were transferred from dark to light there was a stimulation of cell expansion in the primary leaves (Fig.17). The degree of response depended on the age of the plant at treatment. The responses of plants treated on day 2, 4 or 6 were similar to each other and to the cell volume changes of light-grown leaves over the same period. When plants were treated on day 8, 10 or 12 the response in the leaves was much reduced.

In this experiment, plants were grown in the dark cupboard in which the light-leak was developing. The experiment was later
Fig. 15

Changes with time in the fresh weight of the primary leaves of dark-grown plants (closed circles) and of plants transferred to normal light conditions 2, 4, 6, 8, 10 or 12 days after planting (---)
Figs. 16 & 17

Changes with time in the cell number (Fig. 16) and the mean cell volume (Fig. 17) of dark-grown plants (closed circles) and of dark-grown plants transferred to normal light conditions 2, 4, 6, 8, 10 or 12 days after planting (--- - - - )
Fig. 16.

Cell number (millions).

Fig. 17.

Mean Cell volume (cc x 10^9).

Age in Days.

Age in Days.
repeated with plants grown under true dark conditions (Dark room). Plants 4, 6, 8 and 12 days old were transferred from dark to light and the results showed that the response of the primary leaves to the transfer was the same whether plants were grown under true dark conditions, or when the light leak was occurring. In both cases there was an increase in the cell number and mean cell volume of the primary leaves, and in both cases the young leaves responded to a greater degree than did the older ones. For some treatments there were slight differences in the absolute values, but the pattern of response to the light treatment was identical in both cases.

The main conclusion to be drawn from this experiment is that both cell division and cell expansion in the primary leaves of dark-grown Phaseolus plants can be stimulated by transferring plants up to 12 days old to light conditions. In the next experiment, the quantity of white light necessary to stimulate cell division and cell expansion in the primary leaves was studied.

2. The effect on the primary leaves of exposing plants of different ages to a short period of radiation.

It has been shown that transfer from dark to light conditions stimulated leaf growth. This treatment involved exposing the leaves to prolonged periods of illumination. This next experiment was designed to study whether leaf growth could be stimulated by exposing leaves to a short period of radiation.

Plants were grown in darkness, and exposed to white light
for 10 minutes on the 4th, 7th, 10th or 13th day after planting. The plants were returned to dark conditions after illumination. Groups of plants were harvested two and six days after treatment and the fresh weight, cell number and mean cell volume of their primary leaves determined. A group of control plants was grown in darkness throughout the experiment. The white light for the treatment was generated using the light rack described on page 22.

Exposure of dark-grown plants to white light for 10 minutes resulted in increased fresh weight of the primary leaves, irrespective of the age of the plant at the time of treatment (Fig. 18). As in the previous experiment, the effect of the light decreased with the age of the plant at the time of treatment. The effect of this short illumination was much less than the effect of transferring the leaves to normal light conditions (compare with Fig. 9), but the response was nevertheless large and significant.

An increase in cell number of the primary leaves was seen as a result of the 10-minute light treatment (Fig. 19). Here again the effect of the illumination was less on the older plants. This increase in cell number was not as great as that observed when plants were transferred permanently from dark to light conditions (Compare with Fig. 12).

There was little effect of the light treatment on the mean cell volume of the primary leaves (Fig. 20a). There was possibly a small response - all the values for treated leaves were slightly greater than those of the dark controls - but this was not comparable to the large response seen on transfer of plants from dark-
Figs. 18 & 19

Changes with time in the fresh weight (Fig. 18) and cell number (Fig. 19) of dark-grown (closed circles) and of dark-grown plants illuminated with white light on either Day 4, 7, 10 or 13 (other symbols).
Fig. 18. Fresh weight (mg).

Fig. 19. Cell number (millions).

Age in Days.
Fig. 20

(a) Changes with time in the mean cell volume of the primary leaves of dark-grown (closed circles) plants and of dark-grown plants illuminated with white light for 10 minutes, 4, 7, 10 or 13 days after planting.

(b) Changes with time in the mean cell volume of the primary leaves of dark-grown (closed circles) plants and of dark-grown plants illuminated with white light for 5 minutes on days 4 and 5, 7 and 8, 10 and 11, or 13 and 14.
ness to normal light conditions (Compare with Fig.13).

The increase in the fresh weight of the primary leaves as a result of the treatment with white light for 10 minutes was thus due almost completely to an increase in cell number since cell volume was not greatly affected.

Hence though both cell division and cell expansion in the primary leaves can be stimulated by light, it appears that the sensitivities of the two responses differ. Cell division is much more sensitive to light than cell expansion. Since changes in cell number occurred in response to the light treatment which had little effect on the cell volume it appears that light stimulates cell division and cell expansion through mechanisms which differ at least in part.

3. To investigate in detail the effect of a ten minute treatment with white light on the cell number of dark-grown primary leaves.

The previous experiment showed that the main effect of the ten-minute light-treatment on the dark-grown primary leaves was to stimulate cell division. The speed of response to the light-treatment was next studied.

Plants were grown under dark conditions and treated with white light for 10 minutes on either the 6th or the 10th day after planting. Groups of plants were harvested 12 hours, 24 hours, 48 hours and 6 days after treatment along with dark-grown plants, and the cell number of their primary leaves determined.

Fig. 21 shows the increase in cell number of the primary leaves which resulted from the ten minute light-treatment. An
Fig. 21

Changes with time in the cell number of the primary leaves of dark-grown plants and of dark-grown plants illuminated with white light for 10 minutes on day 6 or day 10.
Cell number (millions).

Age in Days.

dark-grown (○).
illuminated for 10 minutes on day 6 (○) or day 10 (□).
increase in cell number of about 50% over the dark control was observed for plants treated on day 6, while for plants treated on day 10 the increase was about 20%.

For plants illuminated on day 6 there was a marked increase in the cell number of the primary leaves 24 hours after treatment. The results indicate that there was an increase in cell number as early as 12 hours after treatment, but as a harvest of dark control material was not made at this time, the significance of the early increase was difficult to assess. However the major increase in cell number occurred between the 12th and 24th hours after treatment. After the rapid rise in cell number from 32 million cells on the day of treatment (day 6) to 53 million cells on day 7 there was a slower rise in cell number to a value of 65 million on day 12.

Ten day old plants also responded rapidly to the ten-minute light-treatment. All the cell divisions initiated by the light-treatment were completed 12 hours after illumination.

For both ages of leaves studied, the response to light was very rapid. By day 10, divisions in dark-grown leaves have normally ceased. The increase in cell number resulting from the light-treatment must therefore be due to induction of divisions in cells which would not divide in darkness. Cell divisions are still occurring in dark-grown leaves on day 6. Because of this, it is not possible to tell whether the light-induced increase in cell number is the result of induction of divisions in cells which would not have divided if left in the dark, or if the effect of the
stimulus is to increase the rate and number of divisions carried out by cells normally dividing in the dark-grown leaf at that age. The main conclusions to be drawn from this experiment are that the response of the cell division mechanism in the primary leaves to the light stimulus is very rapid, and that divisions can be induced in leaves which would not otherwise show mitotic activity.

Up to this point in the investigation, the effects of the different light-treatments have been determined for dark-grown plants of different ages. In many of the following experiments it was not practical to test the effects of the treatments on plants of various ages and plants of one age have been used as the test material.

Six day old plants were chosen for two main reasons. First, leaves from such plants showed the greatest response to the white light-treatments, and secondly, these plants have an upright habit as opposed to the straggly habit adopted by day 8. This second feature facilitated handling of the material during treatment and also led to a more even illumination of the leaves, which helped to cut down the variation between treated plants.

These factors made six day old plants suitable test material for determining the effects of the different treatments on the primary leaves.

It was found that, for dark-grown plants, or for plants illumi-
Fig. 22

Linear regression between fresh weight and cell number of the primary leaves of dark-grown plants and of dark-grown plants which have been treated with white light for 10 minutes.
Regression line for $y$ (cell number) on $x$ (fresh weight)

$y = 7.7101 + 0.1610x$

$r = 0.92$ for 134 df.
nated for ten minutes with white light, where there is little increase in the mean cell volume of the primary leaves over the growing period, that there was a close linear correlation between cell number and fresh weight (Fig. 22). The correlation coefficient was 0.9203

This meant that in experiments where treatments were given which were known not to have a large effect on cell volume, increase in fresh weight of the leaves could be taken as indicating increase in cell number. This fact was used in several of the following experiments. It should be stressed that this approach is legitimate only when it is certain that treatment does not affect cell volume.

So far, work described in this section has shown that cell expansion in the primary leaves is less sensitive to light than is cell division, and it would appear that for marked stimulation of cell expansion to occur, the leaves must be treated either with a large quantity of light or with a light-treatment interrupted by a dark break such as is given in the 12 hours light/12 hours dark regime of normal light conditions. This was examined in the next two experiments.

4. The effect of two short periods of illumination on cell expansion and division in the primary leaves.

The possibility that a dark break in the light-treatment might be necessary for cell expansion to occur, was examined. Dark-grown plants were illuminated with white light for a total period of 10 minutes. This was given as two five minutes periods
Changes with time in the cell number of the primary leaves of dark-grown plants and of dark-grown plants which were illuminated for 5 minutes on days 4 and 5, 7 and 8, 10 and 11, or 13 and 14.
Cell number (millions)

Age in Days.

Dark-grown
Illuminated on days 4 and 5.

- 7 - 8.
- 10 - 11
- 13 - 14
separated by a period of 24 hours dark.

The treatments were given on days 4 and 5; 7 and 8; 10 and 11; or 13 and 14. Plants were harvested on the second and sixth days after treatment and cell number and mean cell volume of the primary leaves determined. Dark-grown control plants were also harvested.

The results of the 5 + 5 minute treatment (Fig. 20b) and the 10 minute treatment (Fig. 20a) were very similar. (The graphs are included together for comparison). There was no increased cell expansion as a result of the 5 + 5 minute treatment, and a similar slight increase resulted from both light-treatments.

The cell number values are shown in Fig. 23. The 5 + 5 minute light-treatment resulted in increases in cell number equal to those seen on exposure to light for 10 minutes (compare with Fig. 19).

5. The effect of different periods of illumination on the development of the primary leaves.

The last experiment suggested that a prolonged period of illumination is necessary to stimulate cell expansion in the primary leaves. The next experiment was designed to determine the length of the period of illumination necessary to produce a marked effect on the cell volume of the primary leaves. The effect of the different periods of illumination on cell number was also studied.

Six day old dark-grown bean plants were exposed to white light for 10 minutes, 1, 2, 6, 12 or 24 hours and then returned to dark conditions. The primary leaves were harvested on day 13.
Cell number values for the primary leaves of 13 day old dark-grown plants and of dark-grown plants exposed to white light on day 6 for either 10 minutes or 1, 2, 6, 12 or 24 hours.

Mean cell volume values for the primary leaves of 13 day old dark-grown plants of dark-grown plants exposed to white light on day 6 for either 10 minutes or 1, 2, 6, 12 or 24 hours.
**Fig 24.**

Cell number (millions).

Period of illumination.

- Dark
- 10 mins.
- 1 hour
- 2 hours
- 6 hours
- 12 hours
- 24 hours

**Fig 25.**

Cell volume (cc. x 10⁹)

Period of illumination.

- Dark
- 10 mins.
- 1 hour
- 2 hours
- 6 hours
- 12 hours
- 24 hours
Cell number and volume were determined and compared with the corresponding values for dark-grown control plants.

All light-treatments stimulated cell division in the primary leaves (Fig. 24), but the results indicate that the longer treatments of 6, 12, and 24 hours resulted in slightly larger cell numbers than did treatment for 10 minutes, 1 hour or two hours. This agrees with the results for the experiment where plants were transferred from darkness to normal light conditions. This experiment showed that when six day old plants were transferred, the final cell number was greater than that found for plants exposed to only 10 minutes light (Figs. 12 & 19). The 10 minutes, 1 hour and 2 hours light-treatments all had similar effects on cell volume and brought about a small increase. The 10 minute light-treatment had a slightly larger effect on volume than usual (Fig. 25 cf, Fig. 20a), but the important feature is that the three treatments all had the same effect. The 6, 12 and 24 hour treatments had increasingly larger effects on cell volume and it would appear that for a marked response in cell expansion to occur in the primary leaves, illumination with white light for 6 hours or longer is necessary.

There are thus two responses of the primary leaves to white light. Firstly, there is the response to a short light-treatment of from 10 minutes to 2 hours in length, which leads to an increase in cell number, and which has only a very slight effect on cell volume. The second response is to periods of illumination longer than 2 hours. This results in a marked
increase in cell volume and in a further, slight, but significant increase in cell number.

6. The effect on the primary leaves of very short treatments with white light.

It has been shown that treatment of dark-grown plants with white light for a short period (10 minutes) stimulates cell division in the primary leaves and that prolonging the treatment to 2 hours had no additional effect. In this experiment, leaves were exposed to periods of illumination shorter than 10 minutes to determine the minimum period of light necessary to bring about this increase in cell number.

Six groups of plants were grown under dark conditions and treated on day 6 as follows:— one group of plants were kept as dark control and the other groups were exposed to white light for either 1, 2, 4, 5 or 10 minutes. After illumination, plants were returned to the dark and they were harvested on day 8. The fresh weights of the primary leaves were determined.

All the light treatments had similar effects on the fresh weight of the primary leaves (Fig. 26). The 1, 2, 4 and 5 minute light-treatments all resulted in an increase in fresh weight comparable to that caused by the 10 minute light-treatment.

Since a short light-treatment has very little effect on the mean cell volume of the primary leaves, any increase in fresh weight is due mainly to an increase in cell number. Hence in these conditions a change in fresh weight is indicative of a change in cell number.
Fig. 26

Fresh weight values of the primary leaves of 8 day old dark-grown seedlings and of dark-grown seedlings illuminated with white light on day 6 for either 1, 2, 4, 5 or 10 minutes.
Fresh weight (mg.)

Period of illumination (mins.)
This experiment thus shows that the cell division mechanism in the primary leaves is sensitive to very short periods of illumination with white light. Whether, as Parkinson (1950) found, plants would respond to illuminations with white light for as brief a period as one second, was not investigated.
Section C: The effect of different light wavelengths on cell division and cell expansion in the primary leaves.

Experiments in Sections A and B have demonstrated that white light can stimulate both cell division and cell expansion in the primary leaves, though probably through two different mechanisms. In this section, the effects on the leaves of short and long periods of irradiation with light of different wavelengths is studied and the role of the different light qualities in controlling the cell division and cell expansion responses investigated.

1. The effect of brief irradiation with red, blue or far-red light on the growth of dark-grown primary leaves.

Cell division in the primary leaves of dark-grown French bean plants can be stimulated by a short irradiation with white light. This experiment was designed to determine which wavelengths of light are active in this stimulation.

Plants were grown under dark conditions and on day 6 were irradiated for 10 minutes with either red, blue or far-red light. Plants were returned to the dark after illumination, and the primary leaves harvested two and seven days after the light-treatment. Fresh weight, cell number and mean cell volume of the treated plants were determined and compared with the corresponding values for dark-grown control plants.

Red light was generated using Cool-White fluorescent tubes and red-glass filters. Cool-White fluorescent tubes and blue-glass filters were used to generate blue light and tungsten fila-
Hemelit lamps (40 watt) with red- and blue-glass filters were used to generate far-red light.

Of the three light qualities studied, only red light had any effect on the fresh weight of the primary leaves (Fig. 27a). Leaves irradiated with red light for 10 minutes showed a large increase in fresh weight. The major part of this increase occurred during the first two days after treatment, followed by a slower increase between days 8 and 13. Irradiation with blue- or far-red light had no effect on the fresh weight of the leaves.

Irradiation with red light resulted in a similar pattern of increase in the cell number of the primary leaves (Fig. 27b), but irradiation with blue- or far-red light had no effect on cell number.

Neither red, blue nor far-red light had any marked effect on the mean cell volume of the primary leaves (Fig. 27c). This agrees with the white-light experiments which showed that a short irradiation did not bring about a large increase in cell volume.

These results showed that when the primary leaves were exposed to a brief irradiation with red light, there was a stimulatory effect on fresh weight which was due mainly to an increase in cell number. Since illumination with red light for ten minutes does not result in any large change in cell volume, the close correlation between fresh weight and cell number observed for dark-grown leaves and for leaves treated with white-light for 10 minutes, (Fig. 22) will also apply here.
Fig. 27

Changes with time in the fresh weight (a) cell number (b) and mean cell volume (c) of the primary leaves of dark-grown plants and of dark-grown plants illuminated for 10 minutes on day 6 with either red, far-red or blue light.
(a) Fresh weight (mg).

(b) Cell number (millions).

(c) Cell volume (cc x 10^9).

- Dark-grown (o)
- Treated with red light (□)
- Blue (○)
- Far-red (△)
Fig. 28

Fresh weight values for the primary leaves of 9 day old dark-grown plants and of dark-grown plants which were exposed on day 6 to illumination for 10 minutes with either red, blue or far-red light.
Fresh weight. (mg.).

- Dark
- Red
- Blue
- Far-red.

Day 9.
As only the red radiation had an effect on the primary leaves, it would appear that it is light of this wavelength which is active in the white light treatments in stimulating cell division.

In an experiment using the cultivar Masterpiece, similar results were obtained. Dark-grown leaves were irradiated for ten minutes on day 6 with red, blue or far-red light and were then harvested on day 9. Red light was generated using Cool-White Fluorescent tubes and one layer of red cellophane; blue light using Cool-White Fluorescent tubes and blue-glass filters and far-red using 40 watt tungsten bulbs with blue-glass filters and one layer of red cellophane. This resulted in higher intensities of red and far-red light than those used in the last experiment (see Figs. 5 & 6).

Results are shown on Fig. 23. Again, only the red radiation had any effect on the fresh weight of the leaves.

2. The effect on the dark-grown primary leaves of following a brief illumination with red light by irradiation with far-red.

Downs (1955) observed that the leaf expansion stimulated by red light did not occur if the red illumination was followed immediately by irradiation with far-red light. This indicated that the phytochrome system was involved in the light-stimulated expansion of the primary leaves of Phaseolus (cv. Red Kidney Bean). The following experiment investigated whether this system was active in the primary leaves of the cultivars Masterpiece and Canadian
Wonder used in this investigation.

Plants were grown for six days under dark conditions. On day 6 plants were split into three groups. One group was left in darkness throughout the experiment. The other two groups were irradiated for 10 minutes with red light, after which one group was returned to the dark while the other was illuminated for 10 minutes with far-red light and then returned to the dark. The primary leaves were harvested two days after treatment and their fresh weight, cell number and mean cell volume determined.

Red light was generated using Fluorescent tubes and red-glass filters, and far-red using 25 watt tungsten bulbs and both blue and red glass filters.

Fig. 29a shows the fresh weight values for three replicate experiments. The solid lines show the results for the individual experiments and the broken lines show the average values for the three experiments. In experiment 1, Warm-White Fluorescent tubes were used as the light source for generation of red light, and in the other two experiments Cool-White tubes were used. This difference in light source had no effect on the results, and there was very close agreement between the three experiments.

There was a marked increase in the fresh weight of the primary leaves as a result of the treatment with red light, but when the red illumination was followed by irradiation with far-red the fresh weight of the leaves did not differ significantly from that of the dark control.

The results in Fig. 29b are cell number values for the first
Fig. 29

Fresh weight (a), cell number (b) and mean cell volume (c) values for the primary leaves of 8 day old, dark-grown plants and of dark-grown plants which have been illuminated on day 6 either with red light for 10 minutes or with red light for 10 minutes followed by far-red light for 10 minutes.

Fig. 30

Fresh weight values for the primary leaves of 8 day old plants of the cultivar Masterpiece which have either been grown in darkness or have been illuminated on day 6 with red light for 10 minutes, or with red light for 10 minutes followed with far-red light for 10 minutes.
Fig. 29

(a) Fresh weight (mg).
(b) Cell number (millions).
(c) Mean cell volume (cc x 10^9).

Fig. 30

Fresh weight (mg).

Day 8.

- Dark
- Red
- Red + Far-red
experiment of the set. They show that the increase in fresh weight resulting from the red light treatment was due to an increase in cell number of the primary leaves and that this increase does not occur if the red irradiation is followed by far-red.

Cell volume values were calculated from fresh weight and cell number values for experiment 1. The data show that neither the red nor the red plus far-red light-treatments had any marked effect on the cell volume of the primary leaves.

A further replicate experiment using the cultivar Masterpiece showed the same results. The plants were treated as before, the only difference being that red light was generated using a single sheet of red cellophane while far-red light was generated using one sheet of cellophane and blue-glass filters. This generated a higher intensity of both red and far-red light (Figs.5 & 6).

Fig.30 shows that as in the last experiment illumination with red light for 10 minutes resulted in an increase in the fresh weight of the primary leaves and that this effect was nullified by subsequent irradiation with far-red light.

These results agree with those of Downs (1955) in showing that the effect of red light on leaf expansion can be reversed by application of far-red light. The data suggest that the phytochrome system is involved in controlling the response of the cell division mechanism to light.
3. The effect on the primary leaves of increasing the time between the red and far-red illuminations.

The stimulatory effect of red light on the primary leaves can be reversed by immediately following the red irradiation by illumination with far-red light. Assuming that the phytochrome system is active in controlling this response, the far-red light will only be effective in reversing the red stimulus if the active far-red absorbing form of phytochrome is present in the leaf and has not decayed or been changed to some other form. The change in effectiveness of the far-red treatment when separated from the red treatment by periods of dark of increasing length can give a measure of the rate of destruction or change of the active phytochrome in the leaves.

Eight groups of plants were grown under dark conditions and were treated as follows on day 6:-- one group of plants was kept in the dark as the control:-- one group was illuminated with red light for 10 minutes (Red cellophane filter and Cool-White fluorescent tubes) and then returned to the dark:-- the other groups of plants were illuminated with red light for 10 minutes and then with far-red light (Red cellophane and blue-glass filters, 40 watt tungsten bulbs) for 10 minutes either immediately or 2, 4, 8, 12 or 24 hours after the irradiation with red light. Plants were kept in darkness between illuminations and were returned to the dark after treatment. All eight groups of plants were harvested on day 8 and the fresh weight of the primary leaves estimated.

The average results of two replicate experiments are shown
Fig. 31

Fresh weight values for the primary leaves of 8 day old dark-grown plants, and of dark-grown plants exposed on day 6 either to red light for 10 minutes or to red light for 10 minutes followed after 0, 2, 4, 8, 12 or 24 hours by 10 minutes illumination with far-red light.

Fig. 32

Fresh weight values for the primary leaves of 6 day old, dark-grown plants, which have been exposed to far-red light for 10 minutes either 13, 29, 39, 50 or 55 hours after planting. Seedlings with, and without, testas were irradiated and appropriate dark control values are shown.
Fig. 31.

Fresh weight (mg.)

- Dark
- Red
- Red + Far-red

Time interval between red and far-red treatments (hours).

Fig. 32.

Fresh weight (mg.)

- Testa removed

Age at time of treatment (hours).

(a) 24, 13, 24, 30, 50, 39, 50, 55

(b) 24, 13, 24, 30, 50, 39, 50, 55
in Fig. 31. The only period of time between the red and the far-red treatments which resulted in a significant decrease in the reversal effect of the far-red light was the 24 hour break. The 2, 4, 8 and 12 hour breaks did not significantly reduce the effect of the far-red light.

4. The role of phytochrome in the normal dark-development of the primary leaves.

The results described so far confirm the suggestion that the phytochrome system is involved in the development of the primary leaves of Phaseolus. It could be that normal development of primary leaves in the dark is controlled by the far-red absorbing form of phytochrome, present in the mature seed as a result of exposure to light during development on the parent plant. If this is so, irradiation of the young seedlings with far-red light should reduce leaf development.

In this experiment, seedlings at different stages of development were irradiated with far-red light. This necessitated more complicated culture techniques than generally used, since seedlings were illuminated at ages when they would normally be below ground level. Seeds were planted on the surface of the sand, watered, covered with moist filter paper and grown under dark conditions. Drying out of the filter paper was reduced by covering the pots with wet blotting paper and a polythene sheet. By using this culture method the young seedlings could be easily illuminated. The plants were exposed to far-red light for ten minutes, 13, 23, 29, 39, 50 and 55 hours after planting. After
irradiation, the seedlings were covered with moist sand and kept in darkness. Control plants were treated in the same manner, but were not exposed to light.

There is a possibility that the thick testas prevent light reaching the interior of the seed. In a second group of plants, therefore, the testas were removed immediately prior to irradiation. The subsequent treatment of these plants was the same as that detailed above. In the control plants the testas were also removed.

Far-red light was generated using light from 40 watt tungsten bulbs filtered through one layer of red cellophane and a blue glass plate. Primary leaves were harvested on day 6 and their fresh weight determined.

The results of the experiment are shown in Fig. 32a and b. None of the treatments of the seedlings with far-red light resulted in a significant reduction in fresh weight of the primary leaves. From this it may be concluded that either the active form of phytochrome is not involved in the dark development of the primary leaves, or, if phytochrome is involved, it must be present in the seedling in a stable form which is not affected by far-red radiation.

5. The effect of prolonged irradiation with red, blue or far-red light on cell expansion and cell division in the primary leaves of dark-grown plants.

It has been shown that in order to stimulate cell expansion in the primary leaves, periods of illumination of longer than two
Fig. 33

Transmission curves for the light source and filters used to generate red, blue and far-red light for the experiment described on pages 51 to 54.
Blue glass filter.

Red

Red + Blue glass filters.
hours must be used. The object of this experiment was to study which wavelengths of light are active in this stimulation. The effect on cell division of prolonged illumination with red, blue and far-red light was also studied.

Four groups of plants were grown in dark conditions until day 6 when they were treated as follows: one group was left in the dark as the control; the other three groups were irradiated continuously for 48 hours with either red, blue or far-red light. After irradiation, the plants were returned to the dark. Plants from each group were harvested on days 8 and 13 and cell number and cell volume of the primary leaves were estimated.

During light-treatment the plants were placed in the light box described on page 23. The box was kept in a growth room at 25°C. Air in the box was changed continuously by means of a small pump (Dymax-Charles Austen). The light source was that normally used in the growth room, i.e. Warm-White fluorescent tubes generating light at about 800 f.c.. These were used with either blue- or red-glass plates for generating blue or red light. For generating far-red light, light from the fluorescent tubes was supplemented with light from two 200 watt tungsten bulbs. Blue and red glass filters were used together in the generation of far-red light. The transmission curves for the red, blue and far-red light used are shown in Fig.33.

This experiment ran concurrently with the experiment investigating the effects of brief illumination with red, blue or far-red light which was described on page 44.
Changes with time in the cell number (a) and mean cell volume (b) of the primary leaves of dark-grown plants and of dark-grown plants exposed on day 6 to continuous illumination with red, blue or far-red light.
EM Cell number (millions) 30

Age in Days. 12

Mean cell volume (cc \times 10^9).

Age in Days. 12

Dark-grown ○
Illuminated with red light □
  blue ○
  "far-red " △
Each of the three light treatments stimulated both cell division and cell expansion in the primary leaves (Figs. 34a & b). There was little difference between the effects of the blue, red or far-red light. The mean cell volume of the treated leaves increased rapidly between days 6 and 8, i.e. during treatment. From day 8 to day 13 cell volume increased at a much slower rate. The cell number of the treated leaves also rose rapidly between days 6 and 8, an average cell number of about 57 million being reached by day 8. After day 8, no further increase occurred. This value of 57 million cells is not as great as that found in leaves grown throughout in dark or in the leaves of plants transferred from darkness to normal light conditions on day 6 (Figs. 16 & 17).

These results substantiate the argument that exposure to prolonged illumination is necessary to bring about a large increase in cell volume of the leaves. Since the treatments with red, blue or far-red light had similar effects on cell volume, it would appear that more than one wavelength can be involved in stimulating cell expansion. It is also interesting to note that when the leaves are exposed to prolonged periods of irradiation, the response of the cell division mechanism is not specific to any one wavelength of light, cell divisions being initiated as a result of exposure to red, blue or far-red light.

The effects on chlorophyll content of the primary leaves of the light-treatments described here, and of the brief-light-
treatments described on page 14, have also been studied. The results are shown in Fig. 14.

Section D: Interrelationships between different organs involved in the response of the leaves to light.

The previous sections have shown that brief illumination of dark-grown plants with white light resulted in stimulation of cell division in the primary leaves. The work in this section covers two aspects of the response to light. These are, first, the location of the site of perception of the light stimulus and second the part played by other organs of the seedling in the response.

1. Determination of the site of perception of the light stimulus.

The object of this experiment was to discover whether for response of the primary leaves to a short white-light treatment to occur, the leaves themselves must be illuminated, or whether the stimulus could be received elsewhere and transmitted to the leaves.

Plants were grown under normal dark conditions. On day 6, the following treatments were carried out: one group of plants was left in the dark and another group was illuminated with white light for 10 minutes and returned to the dark. These two groups of plants acted as controls. Three other sets of plants were illuminated with white light for ten minutes, but before treatment either the primary leaves, or the cotyledons, or the whole
Fig. 35

Fresh weight values of the primary leaves of 8 day old dark-grown plants and of dark-grown plants which have been irradiated with white light for 10 minutes on day 6 either completely (control), or with the cotyledons, the leaves, the leaves and cotyledons together, or the leaves and cotyledons separately covered with aluminium foil during illumination.
Fresh weight (mg)

Cots. Lvs. Lvs+Cots. Lvs+Cots.
together separately. Part covered with foil.

Controls.

Dark-grown

Light treated.
shoot apart from the hypocotyl were covered with aluminium foil to prevent light falling on these parts during illumination. Covering the leaves with foil also involved covering the shoot apex, since this is small and lies between the petioles of the primary leaves in the six-day old dark-grown bean plant. After treatment, the plants were returned to the dark and the foil removed. All five groups of plants were harvested on day 8 and the fresh weights of the primary leaves determined. Two replicate experiments were carried out and the average results are shown in Fig.35.

Since little cell expansion occurs as a result of this light-treatment, the fresh-weight values of this experiment can be directly correlated with cell number values. (See Fig.22).

There was only one group of plants whose primary leaves did not respond to the light-treatment. When the hypocotyl only was illuminated there was no increase in the fresh weight of the leaves. However when the cotyledons or the leaves and apex were masked, the primary leaves did respond to the light. Since a response occurred when the leaves and apex were covered, it seems possible for the light stimulus to be perceived in the cotyledons and transmitted to the leaves. The stimulus could not, however, be effectively perceived in the hypocotyl and transferred to the leaves. For stimulation of cell division to occur in the primary leaves, it would appear that light must fall either on the cotyledons or on the leaves and apex.

Although the whole shoot could be easily and adequately
covered with foil, separate masking of the leaves and cotyledons was not easy. Since it was difficult to ensure that these organs were completely covered and since light leaking under the foil might have masked true responses to treatments, a further treatment was carried out to test this. The primary leaves and the cotyledons on the same plants were covered individually with foil, and the plants were illuminated for ten minutes. The fresh-weight values were determined on day 8; and the results are included with those of the earlier experiment in Fig. 35. For treated plants, the fresh-weight value was smaller than that of the control plants which were completely exposed to light for 10 minutes, and only slightly higher than the value for plants where only the hypocotyl was illuminated.

Since covering leaves and cotyledons independently produced a similar result to enclosing them together, it would appear that this method of covering these organs was adequate. This confirms the previous conclusion that either the leaves and apex or the cotyledons must be illuminated for the primary leaves to respond to the light.

2. The response of excised leaves to light.

Previous results showed that stimulation of leaf growth can be brought about without necessarily irradiating the leaves themselves. This could indicate that the cotyledons are involved in the leaf response and that transport of metabolites from the cotyledons to the leaves might be a necessary part of the response. This was examined in an experiment in which the primary
Fig. 36

Fresh weights of the primary leaves of 6 and 8 day old plants; of 8 day old leaves of dark-grown plants exposed to white light for 10 minutes on day 6; and of 8 day old leaves excised on day 6 and either kept in darkness or illuminated as above.
Fresh weight (mg).


Control. Excised.

- Dark.
- Light treatment.
leaves of dark-grown plants were excised and exposed to a brief illumination with white light to determine whether or not under such circumstances a response to light would occur.

Plants were grown in the dark until day 6 when the leaves of one set of plants were removed and laid on cotton-wool moistened with Hoaglands mineral nutrient solution. Each leaf pair was placed in a separate specimen tube and the tubes were placed in covered spirit pots to prevent evaporation. One set of leaves were kept in the dark together with a group of normal plants as controls and the other set were illuminated for 10 minutes with white light. A group of normal control plants were also illuminated. After illumination leaves and plants were returned to the dark until day 8, at which age they were harvested and the fresh weight of the primary leaves determined.

The results show that the fresh weight of the excised leaves which were kept in darkness was much below that of the dark control leaves (Fig.36). The development of the excised leaves in the dark was less than that for the control leaves, although there was a slight increase over the value for day 6 dark-grown control leaves. This indicates that substances necessary for leaf development are supplied by other parts of the plant. The excised leaves did not respond to the light-treatment. There was no difference between the dark-grown and the light-treated leaves. This is a clear indication that other parts of the plant are necessary to provide materials without which the leaves can not respond to the light stimulus.
3. The involvement of the cotyledons in the response of the leaves to light.

In this experiment, the cotyledons, or part of the cotyledons were removed from the seedlings immediately prior to illumination of the plants. This was done to determine whether substances necessary for the light response to occur are provided by the cotyledons.

Two replicate experiments were carried out, one at 25°C. and one at 21°C. The latter experiment was carried out at this temperature since a preliminary experiment had indicated that the reduced rate of growth at 21°C. would minimise the effect of the absence of the cotyledons in the dark-grown control plants. However this did not prove as effective as had been expected.

At 21°C. the variety Masterpiece was used, and Canadian Wonder was used in the experiment at 25°C. Treatments were given on day 8 to the plants grown at 21°C. (at this stage the plants were morphologically similar to 5-day old plants grown at 25°C.) and on day 6 for plants grown at 25°C.

On the day of treatment, cotyledons or part of cotyledons were removed from 5 groups of plants which possessed either both, one, a half, a quarter, or no cotyledons. Half the plants in each group were then illuminated with white light for ten minutes and returned to darkness. The rest of the plants were kept under dark conditions throughout the experiment. The primary leaves of all groups of plants were harvested two days after treatment. The results are shown in Figs. 37a and b.
(a) Fresh weight values for the primary leaves of 8 day old seedlings which have either been grown in the dark, or have been exposed to white light for 10 minutes on day 6 after removal of either both, $l^1$, $l^2$ or 1 cotyledon.

(b) Results for a similar experiment in which the cultivar Masterpiece grown at $21^\circ C$ was used. Treatments were given on day 8 and leaves harvested on day 10.
Canadian Wonder

- Dark.
- Light treated.

Amount of cotyledon present.

Masterpiece.

Amount of cotyledon present.
In the case of all the dark-grown plants where the cotyledons or part of the cotyledons were missing, the leaf fresh weight was lower than that of the controls. In the absence of the cotyledons, there was no response to the light-treatment; this was observed in both experiments. The results for the experiment carried out at 21°C. showed that as long as part of the cotyledon was present, a response occurred. Even when only a quarter of a cotyledon was present, there was a slight but statistically significant response of the leaves to light. At 25°C. the results were similar, the only difference being that at this temperature the leaves did not respond to light when a quarter of a cotyledon was present. This difference could be due only either to the varietal difference, or to the difference in the physiological ages of the plants at the time of treatment.

When only part of the cotyledons was present the response was never as great as that observed in the control plants with intact cotyledons.

These results show that for the leaves to respond to a brief illumination, some substance or substances must be supplied by the cotyledons.

4. The response of the leaves to 2-chloroethyl trimethylammonium chloride (CCC) in the absence of the cotyledons.

The previous experiment showed that the presence of the cotyledons was necessary for the primary leaves to respond to a brief illumination. It has also been shown (Felippe 1967) that dark-grown primary leaves of Phaseolus show increased cell division
when treated with 2-chloroethyl trimethylammonium chloride applied at $10^{-2}$ M. as a soil drench, although this substance under other circumstances acts as a growth retardant (Tolbert 1960, Sachs & Wohlers 1964 and Zeevaart 1964). In this experiment CCC was used to stimulate cell division in dark-grown leaves in order to determine whether the substances coming from the cotyledons are specifically needed to bring about the light mediated cell division response or whether they are necessary for any cell division response in the leaves.

Two sets of plants were grown under dark conditions at 21°C. On day 8, the cotyledons were removed from one set of plants and half the plants with and half those without cotyledons were treated with 100 ml. $10^{-2}$M. CCC per pot. All plants were kept in the dark until day 10 when the primary leaves were harvested and weighed.

In darkness, when the cotyledons were present, treatment with CCC resulted in an increase in the fresh weight of the leaves (Fig. 38). This has been shown to be an effect on cell division, cell expansion being little affected by the treatment (Felippe 1967), i.e. the response of the leaves to CCC is similar to the response of the leaves to light. When the cotyledons were removed at the time of application of CCC, the leaves did not respond to the CCC. It would appear that the substance or substances transported from the cotyledons to the leaves are needed for an increase in cell division to occur in the leaves and are not involved specifically in the light response.
**Fig. 38**

Fresh weight values for the primary leaves of 10 day old dark-grown plants some of which were grown from day 6 without their cotyledons, and some of which were treated with CCC on day 6; also 2 groups of plants illuminated with white light on day 6, one group of which was also treated with CCC. (For details see text)

**Fig. 39**

Fresh weight values for the primary leaves of 8 day old dark-grown plants and for plants treated with white light on day 6 for 10 minutes, showing the effect on the response to light of removing the cotyledons at different times after light treatment.
Fig 38.

Fresh weight (mg).

Dark grown. Light treated.

+ Cots - Cots + Cots - Cots
CCC CCC CCC CCC

+ Cots - Cots + Cots - Cots
CCC CCC CCC CCC

Fig 39.

Fresh weight (mg).

Dark grown. Light treated.

Control + Cots. Time (hours) between illumination and removal of the cotyledons.

0 3 7 11 24
One further treatment was carried out in this experiment. Normal plants with cotyledons which had been treated with CCC as above were illuminated with white light for 10 minutes on day 8 as also were normal eight day old, dark-grown. The effect on the fresh weight of the leaves was studied. The results which are also shown on Fig. 38 show that the stimulatory effects of light and CCC on the primary leaves are additive. This indicates that light and CCC promote cell division in the leaves acting through mechanisms which differ at least in part.

5. The effect on the response of the primary leaves of removing the cotyledons at different times after illumination.

This experiment was done to investigate the time course of the translocation from the cotyledons to the leaves of the substance or substances necessary for light stimulated cell division in the leaves. To do this, cotyledons were removed at different times after brief illumination of the plants with white light. The period after illumination, over which the cotyledons must be present in order for the leaves to respond, was thus determined.

Six groups of plants were grown in darkness until day 6 when they were treated as follows: half the plants in each group were kept in the dark and half were illuminated for 10 minutes with white light. After illumination, the plants were returned to the dark and the following treatments carried out. In one group of plants the cotyledons were left intact and these acted as dark and light control plants. The other groups of plants
all had their cotyledons removed, either immediately or after 3, 7, 11, or 24 hours. All plants were harvested on day 8 and the fresh weights of their primary leaves determined. In this experiment, an increase in fresh weight is indicative of an increase in cell number since illumination for 10 minutes does not markedly affect cell volume.

The results of the experiment (Fig. 39) showed that for a response to light to occur in the leaves, the cotyledons must be present for longer than 11 hours after illumination. The only group of plants which responded markedly to the light was the group in which the cotyledons were present on the seedlings for 24 hours after light treatment. Even in this case the response was not as great as the response when the cotyledons were left intact throughout the experiment.

There is thus an indication that a slow steady transport of substances from the cotyledons is necessary for response of the cell division mechanism in the leaves to occur. If the supply to the leaves is stopped even as late as 24 hours after light treatment the response of the leaves to light is curtailed.

6. The response of the leaves to light in the absence of the roots.

The previous experiments have shown that some factor or factors must be transferred from the cotyledons to the leaves for a response to light to occur. In this experiment, the roots were removed from the plants at the time of light treatment and the effect of this on the response of the cell division mechanism of the leaves to light studied.
Figs. 40 and 41

Fresh weight values for the primary leaves of 8 day old plants either grown in darkness, or illuminated for 10 minutes on day 6, with, and without, their roots.
Fig 40

Fresh weight (mg).

Control | Roots removed.

Fig 41

Fresh weight (mg).

Control | Roots removed.

Darkgrown

Light treated.
Plants were grown under dark conditions for six days. On day 6 half the plants were cut off at ground level and the cut stems were placed in Hoagland's mineral nutrient solution. Half of the treated and half of the untreated plants were illuminated for 10 minutes with white light and then returned to the dark. The remaining plants were kept in darkness throughout. On day 8 the fresh weights of the primary leaves were determined. (Fig. 40)

Development of the leaves in the dark after removal of the roots was reduced by about 40%, and the response of the leaves to light in the absence of the roots was also reduced. In the control plants the light treatment caused a 70% increase in fresh weight of leaves while in the plants without the roots, leaf fresh weight increased by only 43%. A further experiment by Dale (unpublished), in which plants were treated as above, showed similar results and in this case the response of the leaves when the roots had been removed was even lower (Fig. 41). The effect on the dark control leaves of removing the roots was however less in this experiment.

These results suggest that some substance or substances may be translocated from the roots to the leaves during the development of the seedlings, without which the full response of the leaves to brief illumination will not occur.

7. **The effect on the light response of supplying plants without cotyledons with an external supply of nutrients.**

This experiment studied the light response in the leaves of plants without cotyledons, which had been supplied with an
external supply of minerals, carbohydrates and growth substances. The growth medium used was one on which discs cut from the primary leaves Phaseolus had been successfully cultured and had shown an increase in cell number when grown in white light. (Dale 1966). It was composed of double strength Hoagland's mineral nutrient solution (see p. 15) containing 2/o sucrose, Gibberellic acid (GA3) at 10 p.p.m. and Indol 3-acetic acid (IAA) at 10^-6M. The present experiment investigated whether this same medium could be taken up by the plants and thus overcome the effect of removal of the cotyledons.

The root systems of intact six day old, dark-grown plants were carefully washed free of sand and placed in two, 250ml conical flasks each containing 200ml liquid medium (There were 6 plants to each flask). All the cotyledons were removed. Half the plants were then illuminated for 10 minutes with white light and returned to the dark. The other half was kept in darkness throughout the experiment. All the primary leaves were harvested of day 8 and their fresh weights determined.

The fresh weight results (Fig. 42) show that there was no effect of light on the leaves of the illuminated plants. Hence all the factors necessary for a response to brief illumination to occur cannot have been present. Thus, either the medium was not taken up by the plants, or if it was taken up, the substances in the medium alone are not adequate to allow light induced stimulation of cell divisions to occur in the primary leaves in intact plants.
Fig. 42
Fresh weight value for the primary leaves of 8 day old plants without cotyledons, which were placed in nutrient solution (see page 64) on day 6, and were exposed to white light for 10 minutes. The dark-control value is also given.

Fig. 43
Fresh weight values for the primary leaves of 8 day old, dark-grown plants with, and without, cotyledons, which were transferred to continuous illumination with white light on day 6. Appropriate dark-control values are also shown.
8. The effect of prolonged illumination with white light on the leaves of plants without cotyledons.

The previous results showed that there was no effect of brief irradiation on the primary leaves of plants without cotyledons. The final experiment in this section investigated the response of the primary leaves to prolonged illumination with white light in the absence of the cotyledons.

Two groups of plants were grown under dark conditions for six days. On day 6, the cotyledons of one group of plants were removed and half the plants in each group were transferred to a growth room and exposed to continuous illumination for 48 hours. The other plants were kept in complete darkness throughout the experiment. Plants were harvested on day 8 and fresh weights of the primary leaves determined.

The results (Fig. 43) show that there was a marked response to the light treatment in plants without cotyledons. The final fresh weight of the leaves from plants without cotyledons was not as great as that found in plants with intact cotyledons, but it was very much greater than the dark control values for plants both with and without cotyledons. It was also observed that the light-treated leaves had greened.

In this experiment, leaf growth was not differentiated into cell division and cell expansion. Since the leaves greened and presumably began to photosynthesize during the period of illumination, it is difficult to distinguish between a straight morphogenic response of the leaves to light and secondary effects.
resulting from photosynthetic activity in the leaves. To do this it would be necessary to employ some photosynthetic inhibitor. However, even if the expansion of the leaves is a secondary effect, the fact that greening occurred in the leaves, indicates that the metabolites necessary for this must have been available within the plant even in the absence of the cotyledons. In this connection, it should be remembered that for young leaves, up to 60% of the protein may be contained within the chloroplasts (Zucher and Stinson[162]).

Section E. Biochemical changes which occur in the leaves as a result of light treatments.

In previous sections, the effect of light treatments on the cell number and volume of the primary leaves has been studied. In this section, biochemical changes occurring in the leaves as a result of various light treatments are reported.

1. Chlorophyll content.

The chlorophyll content of the leaves was studied in plants grown in the dark and in plants exposed to brief or prolonged illumination.

Two groups of six day old, dark-grown plants were treated as follows: in the first group, plants were illuminated for ten minutes with red, blue or far-red light, while in the second group, plants were illuminated with blue or far-red light for two days. All plants were returned to the dark after treatment.
Fig. 44

Changes in the chlorophyll content of primary leaves of dark-grown plants which, on day 6, were either illuminated for 10 minutes, or were transferred to conditions of continuous illumination with red, blue or far-red light. Control values for dark-grown leaves are also shown.
OD units leaf pair.

Age in Days.

- Dark grown.
- Illuminated with red light.
- " blue "
- " far-red "
- Continuous illumination from day 6.
Leaves were harvested two and six days after the commencement of treatment and dark control leaves were harvested on days 4, 6, 8 and 12. (This is leaf material from the experiments described on pages 44 and 51 which investigated the effects of short and prolonged illuminations on the cell number and cell volume of the leaves. Chlorophyll was extracted from all leaves using the method described on pages 19 and 20. Results were expressed in OD units/leaf pair (in 10ml.) and are shown in Fig. 44.

It was found that while none of the short light treatments promoted synthesis of chlorophyll in the leaves, prolonged treatment with light did. The blue and red wavelengths were most efficient, both wavelengths having similar effects on chlorophyll synthesis, this agrees with the results of Virgin (1958). A relatively small amount of chlorophyll was however formed in the leaves as a result of the prolonged treatment with far-red light.

Similar results were seen when plants were illuminated with white light. There was no synthesis of chlorophyll in the leaves as a result of illuminating six-day old dark-grown plants with white light for ten minutes.

In leaves exposed to light for ten minutes, there is no chlorophyll synthesis, hence all the changes occurring in the leaf as a result of this treatment must be independent of the photosynthetic mechanism. However, the results indicate that in plants exposed to prolonged illumination, photosynthesis may occur, thus some of the changes observed in leaves which have been exposed to prolonged irradiation may be secondary effects resulting from photosynthetic activity in the leaves.
2. Nucleic acid content.

Changes with time in the DNA and RNA content of dark-grown leaves was also investigated and the effect on the DNA and RNA content of the leaves of treating them with white light studied.

Dark-grown leaves were harvested on days 4, 6, 8 and 13 and the leaves of dark-grown plants which had been treated with white light for ten minutes on day 6 were harvested on days 8 and 12. DNA and RNA content of the leaves was estimated using the method described on page 20.

The results which are shown in Figs. 45 and 46 are average values obtained from two replicate samples of leaves. Each sample contained at least five leaves. The number of leaves in the sample varied, with the size of the leaves, fourteen leaves were used in the sample of four-day-old dark-grown leaves.

(i) RNA content.

As can be seen in Fig. 45a, the RNA content of the dark-grown leaves rose until day 8, the greatest rate of increase being shown between days 4 and 6. After day 8, the RNA content remained constant. The light treatment resulted in a marked rise in RNA content per leaf between days 6 and 8 followed by a slower increase between days 8 and 13.

When RNA content was converted to a per cell basis a similar pattern was observed. (Fig. 45b) In the dark-grown leaves, RNA content per cell rose until day 8 and then remained constant. The 10 minute light treatment resulted in an increase in RNA per cell to a value slightly above that of the dark-grown leaves.
Fig. 45

Changes with time in the RNA content of the primary leaves of dark-grown plants (closed circles) and of dark-grown plants illuminated with white light for 10 minutes on day 6 (open circles). Results are expressed per leaf pair (a) and per 50 million cells (b).
Fig. 46

Changes with time in the DNA content of the primary leaves of dark-grown plants (closed circles) and of dark-grown plants illuminated with white light for 10 minutes on day 6 (open circles). Results are expressed per leaf pair (a) and per 50 million cells (b).
(ii) DNA content.

In the dark, DNA content per leaf increased rapidly until day 6 and then more slowly between days 6 and 8 (Fig. 46a). From day 8 to day 13, there was a marked decrease in the DNA content of the leaves. Treatment of the plants with white light for ten minutes resulted in rapid increase in the DNA content of the leaves above that found for the dark control. There was a rapid rise in DNA between days 6 and 8, but between days 8 and 13 there was a decrease in DNA content similar to that found for dark-grown leaves.

If DNA content per cell of dark-grown leaves is examined (Fig. 46b), it may be seen that the average DNA content rose rapidly between days 4 and 6, less rapidly between days 6 and 8 and then decreased between days 8 and 13. The increase in DNA content per cell occurs during the period of active cell division in the leaves and is presumably due to doubling of the DNA level in those cells about to divide where DNA content is raised from the 2C to the 4C state. The slight increase in average DNA per cell between days 6 and 8 is possibly due to cells reaching the 4C condition but not dividing. Cell division in the leaves is decreasing over this period. (see Fig. 12).

The observed decrease in the DNA content per cell of the dark-grown leaves from day 8 onwards can not be due to cell division reducing 4C nuclei to the 2C state since active cell division is not occurring in the leaves over this period. The decrease could be due to death and loss of contents of cells in
the leaves reducing the average amount of DNA in the cell (this assumes that the cells which have lost their contents are counted in the estimation of cell number). A further possibility is that DNA is lost from living cells reducing their nuclei from the 4C to the 2C condition.

In the illuminated plants, the average DNA content per cell dropped rapidly between days 6 and 8 indicating that cells in the 4C condition in the dark-grown leaves had been reduced to the 2C state on stimulation of cell division by the light treatment. This substantiated the argument that the high DNA content per cell observed on day 8 in the dark-grown leaves is due to cells being in the 4C condition.

The continued decrease, from day 8 onwards in the DNA per cell of the light-treated leaves (i.e. over the period when most cell divisions have stopped) could be explained by the same arguments as were used to explain the decrease in DNA per cell observed to occur between days 8 and 13 in the dark-grown leaves.

3. **Protein content.**

Changes with time in the protein content of dark-grown and light-treated leaves were investigated in an experiment similar to that in which DNA and RNA were estimated.

Dark-grown leaves were harvested on days 4, 6, 8 and 13 and the leaves of dark-grown plants which were illuminated with white light for 10 minutes on day 6 were harvested on days 8 and
Fig. 47

Changes with time in the protein nitrogen content of the primary leaves of dark-grown plants (closed circles) and of dark-grown plants illuminated with white light for 10 minutes on day 6 (open circles). Results are expressed per leaf pair (a) and per 50 million cells (b).
Fig 47a

Protein nitrogen/leaf pair.

Protein nitrogen/50 million cells.

age in days.
13. The leaves were extracted with boiling 80% ethanol and their nitrogen content estimated using the method of Conway described on page 21. Two replicate samples of leaves were used. The number of leaves in each sample varying from one to three depending on the size of the leaves used. From these results, the protein content per leaf pair and per cell was calculated in µg protein nitrogen following the method described on page 21 using the DNA and the RNA values obtained in the experiment previously described.

In the dark there was a rapid increase in the protein content of the leaves up to day 8 (Fig. 47a) after which time the rate of increase declined, this reduction coinciding with the time at which cell divisions were stopping in the leaves (see Fig. 12).

As a result of illumination with white light on day 6 there was a marked increase in the protein content of the leaves. The major part of the increase was over within two days of the light treatment, i.e. by day 8, though there was a slight increase between days 8 and 13. Some of this increase may be due to the formation of plastid proteins, Nego and Jagendorf (1961) have shown that on illuminating dark-grown plants of Phaseolus vulgaris (Black Valentine) for periods from one to 12 hours, there is a marked increase in protein synthesis in the plastid.

If the protein content of the leaves is estimated on a per cell basis, a different picture is observed. (Fig. 47b). The protein content per cell of the dark-grown leaves decreased between days 4 and 6 and then increased between days 6 and 13. The de-
crease in protein content of the cell corresponds with the period of rapid cell division in the leaves (see Fig. 12) while the increase in protein content per cell corresponds with the period during which cell divisions are declining and ceasing in the leaves.

As a result of illumination on day 6, there was an increase in the protein content per cell. It appears from the graph that this increase stopped after day 8, however, since there was not sufficient material to permit a statistical analysis it is possible that this flattening of the curve between days 8 and 13 is not a real effect and that the difference between the dark- and light-treated material is not statistically significant.

Thus it would appear that while in dark-grown leaves accumulation of protein in the cells does not occur until cell division ceases, in light-treated leaves accumulation of protein in the cells can occur concurrently with cell division. As mentioned previously, this may be connected with synthesis of plastid protein.
IV - DISCUSSION

A. Seedling development.

Considering development of the *Phaseolus* seedling as a whole, we see that light controls elongation of the hypocotyl, expansion of the primary and trifoliate leaves, development of the floral primordia, and elongation of the internodes. (See Fig. 6) The response to light varies in different parts of the plant; while growth of the hypocotyl is retarded by light, growth of the leaves, stems and floral primordia is promoted by light.

In the varieties of *Phaseolus* studied here, light during germination is not necessary for the initiation of foliar and floral primordia. The same number of leaf primordia are produced in the dark as in the light, though at a slightly lower rate. Light is however necessary for their subsequent development, and for the development of the primary leaves. These results agree with those of Thomson and Miller (1961) who, working with peas, observed that normal leaf primordia were produced in dark-grown plants although at a slightly slower rate than that observed for plants grown in 16 hour days of white or red light. Similar results were obtained by Thomson (1950) using *Avena* seedlings. Butler and Lane (1959), using broad bean seedlings, showed that although the form of the primordia was the same in
darkness as in light, the rate of production was slower. Only nine leaf primordia were produced by day thirteen in the dark as opposed to eleven in the light. However, Mohr and Pinnig (1962) maintained that the rate of production of primordia of Sinapis alba and the number produced was controlled by light, partly through the phytochrome system and partly through the high energy reaction system.

It is difficult to compare leaf primordium production in varieties of Phaseolus such as Canadian Wonder, which show a determinate growth habit with formation of a small fixed number of leaves on the main stem, with species like Vicia and Pisum where large numbers of leaves were produced. Where many leaves are produced, it is possible that nutrient supply will limit the number of primordia produced in dark-grown plants; indeed the work of Thomson and Miller indicates that the difference in primordial number between dark- and light-grown peas increases with age. In the experiment with Sinapis however (Mohr & Pinnig, 1962), the dark effect was observed early, in the formation of the first three primordia. By day seven, an average of 1.4 primordia had been produced in dark-grown plants as opposed to 3.2 found in the light.

If the phytochrome system is involved in the production of primordia, it is possible that the variation in the different species could be due to the differences in the effect of light on the developing seeds. It could be that in seedlings which produce many primordia in the dark, there is some active phytochrome (Pfr) present in the seed as a result of illumination of
the seed on the plant during development, and it is this which stimulates primordial production. If \( P_r \) was absent, or present only in small quantities in *Sinapis*, production of primordia without the stimulation of light would be reduced.

If active phytochrome in the seed is responsible for seedling development and for the production of primordia, it is possible that illumination of the young seedlings with far-red light might prevent it. However, irradiation of young *Phaseolus* seedlings with far-red light did not apparently affect the development of the seedlings. Primordia were not counted in this experiment, but the development of the primary leaves was unaffected by the treatment and the appearance of the seedlings was no different from that of the dark controls. From this we may conclude that either phytochrome is not involved in the dark development of the *Phaseolus* seedling, or if it is involved, it must be present in the seedlings in a form which is not photo-reversible.

Thus in *Phaseolus*, light during germination is not necessary for production of leaf primordia, but it is necessary for the subsequent development of the trifoliolate leaves and for the development of the primary leaves.

B. **Effects of light on the primary leaves.**

Increases in area, fresh weight, and dry weight of the primary leaves of light-grown *Phaseolus* seedlings over the first
six days after planting are due to both cell division and cell expansion; cell expansion keeping pace with cell division so that the increase in average cell volume is relatively small. From day 6 onwards, as cell divisions cease, cell expansion plays an increasingly important role in the growth of the leaf, and cell expansion alone is responsible for increases in leaf area from day 8 onwards. This agrees with work by Dale (1964b).

There are several points of similarity in the development of the primary leaves in the dark and in light. Area, fresh weight and dry weight increase exponentially over the period day 4 to day 8 in darkness and in light, but the rate of increase is lower in darkness, and from day 8 onwards there is no further increase in the size of the dark-grown leaves. Cell divisions occur over the same period in the dark as in light, but in the dark, the final cell number is about half that found in the light, indicating either that the rate of division is greater in the light or that more cells must be dividing. As in the light-grown leaves, there is a comparatively small increase in cell volume over the first six days from planting and the rapid increase in volume from day 6 onwards which is characteristic of light-grown plants does not occur, cell volume remaining constant. Growth of the leaves in darkness is thus due to both cell divisions and limited cell expansion, and is equivalent to the first phase of growth observed in light-grown plants.

Light stimulated expansion of leaves has been observed by many other workers, among them Priestly (1925), Trumpf (1924a, 1924b), Butler (1963), Mohr and Pinnig (1962), Parkinson (1950)
and Downs (1955), but of these, only Parkinson and Butler have differentiated the effect of light into effects on cell division or on cell expansion. Their results showed that in Pisum, and Vicia, cell division and cell expansion are both stimulated by light agreeing with the results obtained here for Phaseolus.

Growth of the primary leaves of Phaseolus can thus be categorised under two headings. First, there is that cell division and cell expansion which can occur in the dark, i.e. the basic background divisions which give a final cell number of 30-40 million cells while cell expansion keeps pace with cell division, to result in a comparatively small increase in mean cell volume. Secondly, there is that cell division and expansion which occurs only on illumination of the leaves. This accounts for half of all the divisions in the light-grown leaves, and for the major increases in cell volume. It is the mechanism involved in the light-stimulated cell division and cell expansion which have been investigated here.

From the results which have been reported it can be seen that the mechanism stimulating cell divisions is much more sensitive to light than that which leads to large increases in cell volume. Illumination of dark-grown leaves with white light for one minute stimulated cell divisions in the leaves, but periods of illumination of six hours or longer were needed before a marked increase in cell volume was observed. The work of Butler (1963) is in agreement with these results. As discussed earlier he observed that in Vicia leaves, cell division was more sensitive to light than was cell expansion. The results suggest that
two different mechanisms are involved in controlling the cell division and cell expansion responses in the leaves. This is at variance with conclusions of Parkinson (1950) who claimed that illumination of dark-grown pea plants with white light for one second, produced an increase in leaf cell volume. However, as mentioned on page 3, Parkinson did not obtain direct estimates of cell volume.

While prolonged illumination of the leaves is necessary for large increases in cell volume, some cell expansion does occur in the dark and in dark-grown leaves which have been exposed to a brief irradiation. This is the expansion which results in the two- to three-fold expansion of the dark-grown cells during the period of cell division in the leaves. Since this expansion can occur in the absence of light, the factors controlling it are probably different from those operating in the massive expansion resulting from prolonged illumination.

In the light-grown plants, the large increases in cell volume observed from day 6 are presumably due to the emergence of the leaves above soil level and their exposure to high levels of radiation from day 5. The light to which the leaves had been exposed up to day 5 would only be that which filtered through the sand and this is presumably not sufficient to stimulate large increases in cell volume. It could be argued that since in the light-grown leaves large increases in cell volume do not occur until cell divisions cease such increases in volume are not possible in leaf cells which are still dividing. However, in the experiments in which six-day old dark-grown plants were exposed to
prolonged illumination with either white or coloured light, cell division and increase in cell volume took place over the same period, suggesting that both can occur concurrently in the leaves. Sunderland (1960) investigating cell expansion and cell division in Lupin and Sunflower leaves observed that cell division and increase in cell volume occurred concurrently over long periods. It would seem that it is the exposure of the *Phaseolus* leaves to prolonged light from days 5 and 6 onwards which results in the observed massive increases in cell volume, and that these increases are possible even though cell division is proceeding. This is confirmed by results (Dale 1966) for discs cut from young *Phaseolus* leaves, (incubated on a medium containing sucrose) which showed appreciable division and cell expansion to occur side by side.

Investigation of the wavelength requirement for stimulating cell expansion in dark-grown leaves showed that blue, red and far-red light have similar effects on cell expansion. This response of the cell expansion mechanism to light depended on the age of the plant at the time of illumination. When dark-grown plants, eight days old or older, were transferred to normal light conditions, the effect of the light on cell expansion was less than that observed in the younger plants, and decreased progressively with the increasing age of the plant. This could be explained in several ways. The decreasing response could be due to changes in the ageing cells, such as, for example, changes in the cell wall plasticity of the older leaves. Again the
ageing response could be due to permanent changes in the composition of cell proteins which preclude further growth. Other possibilities which would also bring about this ageing effect are, that the mechanism which receives the light stimulus may change in the older leaves, or that in the older plants, materials supplied by the cotyledons which are necessary for cell expansion become limiting.

When considering this last possibility, it must be kept in mind that in the dark, the reserves in the cotyledons are rapidly diminished. In this investigation, cotyledons dehisced by day 11 or 12, Opik (1966) has observed that the reserve material in the cotyledons of Phaseolus grown at 25°C are almost completely digested by day 8. This is confirmed by Dale (unpublished). These results might substantiate the argument that those substances necessary for cell expansion which are provided by the cotyledons become limiting after day 8; however, further evidence disagrees with this. In the experiment, described on page 65 in which six-day old dark-grown plants without cotyledons were transferred to continuous light, marked expansion of the primary leaves occurred which could not have been due solely to cell division. Thus it would appear that increase in volume of the cells of the primary leaves can take place without substances being supplied by the cotyledons. This is confirmed by work done by Wheeler (1966). He observed that removal of the cotyledons from five-day old light-grown plants of Phaseolus had only a slight effect on the mean cell volume of the leaves.
These results suggest that in the older leaves, the reduced response of the cell expansion mechanism to light is a result of changes occurring in the cells of the leaves themselves, possibly of the type already mentioned.

It is difficult to specify what these changes in the cells of the leaves might be, since the mode of action of the prolonged light stimulus is unknown. It is possible that light might act through the HER as described by Mohr and co-workers (see page 9) i.e. through phytochrome action; or through the mechanism proposed by Evans et al. (1965) (see page 10) which involves both phytochrome action and the supply of some other material necessary for the response to phytochrome to occur. It is impossible on the present evidence to say which is correct. However, the lack of response of the cell expansion mechanism to a brief illumination with red light which activates the phytochrome mechanism, suggests that some other factor is involved. It would be interesting to see whether, after a period of illumination with far-red or blue light, additional expansion could be stimulated by a brief illumination with red light acting through the phytochrome system. This would be similar to the situation found by Evans et al. in lettuce seedlings, and would indicate that both phytochrome and some other substance were involved in the response. If Mohr's theory (1966) that the HER acts solely through phytochrome is applied here, then for the reasons already discussed in the introduction, it would be expected that far-red and blue light would be more effective than red, but this is not so. This further suggests the involvement of some
other factor.

The results indicate that continued illumination of the plants may be necessary for cell expansion, since on returning plants to the dark after illumination for two days with red, blue or far-red light, cell expansion ceased. This suggests that the continued production of some material as a result of illumination may be involved in the response. Dale (1966) using leaf discs cut from the primary leaves of light-grown *Phaseolus* plants observed that in alternating periods of 12 hours dark and 12 hours light, cell expansion occurred in the dark at the same rate as in the light. He has also observed that on transfer of light-grown plants to darkness, cell expansion ceased and suggests that although cell expansion may occur in the dark for periods up to 12 hours, after this time it is possible that further increase in cell volume will not occur unless more materials are provided by the light reaction. This would agree with the results obtained in this investigation.

The interpretation of the effects of prolonged light is further complicated by the facts of formation of chlorophyll and the possible commencement of photosynthetic activity in the leaves. This may be involved in the response of the leaves to light. To investigate this it would be necessary to use photosynthetic inhibitors. Evans et al (1965) used the photosynthetic inhibitor 3-(3,4 dichlorophenyl)-1 dimethyl urea (DCMU) in their investigation of light-controlled expansion of lettuce hypocotyls and showed that the inhibitory effect of exposing the
seedlings to white light for ten minutes was unaffected by the presence of DCMU. They state that this indicates that the light reaction is independent of photosynthesis. In the present investigation, the fact that response to prolonged far-red light was as great as that observed in red or blue light suggests that a direct photomorphogenic effect of light may be involved. In far-red light, chlorophyll formation was greatly reduced, and if photosynthesis did occur in the leaves in the course of the experiment, the activity in far-red light would be much lower than that found in red or blue light. Photosynthetic activity is greatest in red and blue wavelengths although far-red light is not completely inactive. Whatley (1965) quotes work by Tagawa and co-workers which shows that light at wavelength 708 nm can bring about cyclic photophosphorylation producing only ATP, and that photoreduction of TNP will occur at this wavelength, not with water as usual, but when electrons are supplied by the ascorbate dye couple. Thus the possible involvement of photosynthetic activity in the cell expansion response to light cannot be excluded. Dale, using the leaf disc technique referred to previously, has studied discs cut from ten day old plants where the leaves are active photosynthetically and where massive increase in cell volume is occurring. His observations led him to suggest that sucrose, formed as a product of photosynthesis, is not involved with the pathway concerned with cell expansion in the leaves. Thus if photosynthesis is involved in the response of the cell expansion mechanism to light, it
is probably not through a direct supply of cell wall materials, at least for young leaf tissue up to ten days old. Later work (Dale 1967) has given further support to this idea.

Cell division in the leaves can be categorised as that which takes place in the dark, and that which occurs only on stimulation by light. Results have shown that it is possible to stimulate cell division in the leaves by exposing them to white light for a period as brief as one minute. Similar results were obtained by Parkinson (1950) who observed increases in cell number of dark-grown pea leaves as a result of exposing them to white light for one second on two consecutive days. In Phaseolus, increasing the period of illumination from one minute to two hours had no increased effect on cell division. Parkinson showed that increasing the daily period of illumination from one second to three minutes had an increased effect on cell division in the leaves of Pisum, but subsequent increase of the period of illumination from three to twelve minutes had no further effect. It appears that a certain amount of illumination, which can be provided in one or several short periods, will saturate the reaction after which further illumination, unless it is prolonged for several hours, has no effect. The slight difference between the results of Parkinson and those quoted in this investigation could be due to species differences, or to the much lower intensity of light used by Parkinson, or to the difference in
the number of illuminations to which the plants were exposed.

The data show that cell division induced in the primary leaves of *Phaseolus* by brief illumination are stimulated by way of the phytochrome system. Brief illumination with red light resulted in an increase in cell number, but this effect was nullified if red irradiation was followed by irradiation with far-red light. Brief illumination with far-red light alone, or with blue light, had no effect on cell division in the leaves. The work of Downs (1955) examining the effect of different wavelengths of light on expansion of the primary leaves of *Phaseolus* (see page 6) agrees with this, as does the work done on etiolated *Phaseolus* leaf discs by Liverman et al. (1955), Miller (1956), and Powell and Griffith (1960). Klein and Wansor (1963) claimed that brief irradiation with blue light had a stimulatory effect on cell division in discs cut from etiolated *Phaseolus* leaves. They also claimed that effects of both blue and red light could be reversed by exposure to far-red radiation. In Klein and Wansor's experiments, illumination with blue light was for periods of thirty minutes or less and it is unlikely that the effect of prolonged light is involved. This effect of blue light cannot readily be reconciled with the results obtained here. Possibly there is some difference in the response to light of leaves attached to the plant and leaves in culture.

The investigation by Parker et al. (1949) into the effects
of different wavelengths on the elongation of pea leaves is also not in complete agreement with the results obtained here. They observed that on illuminating plants daily for five days for periods of five minutes, red light at 670nm was most effective in promoting leaf elongation but observed also that far-red light at 730nm had a market effect over that of the dark control. It is possible that daily illuminations may result in a different effect from that produced by a single brief illumination and this might also explain the slight differences between the present work and the results obtained by Parkinson which were discussed earlier. Biebel (1942) working with Phaseolus states that a maximum morphological response to light was observed in plants irradiated two to three times daily. However our data show that illumination for five minutes with white light on two consecutive days has no more effect on cell division in the leaves than does one illumination for ten minutes; but it is possible that more frequent or a larger number of illuminations could have an additional effect.

When the effect of prolonged illumination on the cell division mechanism of the primary leaves was studied, it was found that periods of illumination longer than two hours had an additional stimulatory effect. This suggested that light can stimulate cell division in two ways. The first way involves action through the phytochrome mechanism, cell divisions being
triggered off by a brief illumination - this is an all-or-nothing type of response. The second way involves exposure to prolonged illumination, blue, red and far-red light all being equally effective in promoting cell division. There is no evidence as to how the mechanism of this action works, and some of the arguments that were applied in considering the mode of stimulation of cell expansion by prolonged exposure to light might be applied here. The possibility of photosynthesis being involved in the leaf response cannot be ignored. However Gregory (1928) states that the action of light in controlling the development of Cucumis leaves is not inconsistent with a hypothesis of a master photochemical reaction independent of carbon assimilation leading to the formation of a substance which is directly involved in leaf expansion.

The intensity of the light appears to be important in the response of the cell division mechanism. This is indicated by the fact that transfer to normal light conditions, where the light intensity was higher, was more effective than prolonged illumination with red, blue or far-red light. The length of the period of exposure to light also appears to be important in the response since increasingly longer periods of illumination from six hours onwards had increasingly larger effects.

In considering the effects of prolonged illuminations, a complication exists in that the energies of light given at the three wavelengths were not identical. However, the smallest light dose in terms of absolute energy was that of red light which
gave an effect as great as that observed using higher energies of far-red and blue light. In experiments investigating the effects of brief light treatments, differences in the intensities of the light generated at the three wavelengths studied were relatively unimportant as this response has been shown to be saturated by a small dose of radiation.

When the response of the leaves to a brief irradiation was studied in greater detail, it was found that it was not necessary for the leaves themselves to be directly illuminated. Cell division in the leaves resulted if the hypocotyl and the cotyledons were illuminated, though not if the hypocotyl alone was irradiated. This differs in part from results obtained by Biebel (1942) who observed that although all parts of the Phaseolus seedling could perceive the light stimulus, the leaves and the growing point were most sensitive. The results of work carried out by Briggs and Siegelman (1965) showed that in Phaseolus the distribution of phytochrome in the seedlings roughly paralleled the sensitivity to red light and that the highest concentration was found in association with meristematic tissue.

The ability of the cotyledons to receive the light stimulus suggested that they might be involved in the light response. The lack of response of the detached leaves to light indicates that materials supplied from other parts of the plant are necessary for the response of the cell division mechanism in the leaves to light. It was found that on removal of the roots, the response of the leaves to light was reduced, while removal of the cotyle-
don s completely prevented the light stimulated increase in leaf cell number. This suggests that substances necessary for light-stimulated cell division s are provided both by the coty-ledone and the roots. The rôle of the roots was not investigated further, but it is possible that it may involve the production of some horm one such as gibberellic acid. Phillips and Jones (1964) and Carr and Reid (1964) all showed that the roots are a site of gibberellin synthesis and Dale (1966) has shown that gibberellic acid has a marked effect on the development of discs cut from Phaseolus leaves and grown in culture.

While removal of the cotyledons completely prevented the response of the leaves to light, removal or part or the cotyle-dons reduced the response, but the leaves did give at least a slight response to light even when only a small part of a single cotyledon was present. It should be noted that the normal dark development of the leaves was also reduced in the absence of the cotyledons. These results are indirect evidence that the degree of the response of the leaves to light may depend on the amount of materials provided by the cotyledons. The removal of the cotyledons at different times after illuminating the plants produced results indicating that these materials must be supplied over a prolonged period for the response to occur. If the coty-ledons were present for only twelve hours after light treatment, the cell division m echanism did not respond. Even when the cotyledons were present for a period long enough for cell divi-sion to be stimulated (twenty four hours) there is evidence that
cell divisions ceased on removal of the cotyledons, since the fresh weight of these leaves was less than that of control leaves. This result agrees with that for the experiment in which plants were illuminated with red light, followed after various intervals by far-red light. The data showed that for the reversibility of the red light effect by far-red light to be significantly reduced, the red and far-red illuminations must be separated by more than twelve hours, although there was an indication that periods less than this had a slight effect. This suggests that much of the Pfr may not be effective until twelve hours after light treatment; this could explain why the cotyledons have to be present for longer than twelve hours for the response to light to occur. It is relevant here that detailed investigation of the pattern of division in response to light on day six showed that, although some divisions may have occurred by twelve hours after illumination, the majority of the light-stimulated divisions occurred between twelve and twenty-four hours after illumination.

Initiation of cell divisions in the leaves by means of a brief light treatment (one to ten minutes) appears to be an 'all-or-nothing' reaction, since increasing the period of illumination to two hours had no additional effect, nor was there any additional effect of giving a total of ten minutes illumination as two five minute periods on consecutive days. We may thus consider that light acting through the phytochrome
system triggers off a series of reactions in the leaf, which lead to cell division and which require continued production of some substance or substances from the cotyledons. The question which must be asked at this point is whether the Pfr formed as a result of the light treatment has a direct effect on the cotyledons, resulting in the production of some factors necessary for cell division in the leaves, or whether formation of Pfr involves a reaction in the leaves which leads to cell division only if other necessary materials are supplied by the cotyledons.

In the absence of cotyledons, the leaves do not respond to CCC, which normally stimulates cell division (Felippe 1967). The effects of CCC and light on dark grown leaves are additive indicating that they exert their effect on cell division through pathways which differ at least in part. The need for cotyledons, for both the CCC and the light response, indicates that substances provided by the cotyledons are required for cell division to occur in the leaves irrespective of the mode of stimulation. The fact that normal development of leaves in the dark is reduced when both, or part, of the cotyledons are removed substantiates this argument.

The conclusion that substances provided by the cotyledons are required for all cell divisions in the leaves does not rule out the possibility that the phytochrome-mediated response might involve some changes in the cotyledons. Investigations by Felippe (1967) in-
to the effect of CCC on the development of Phaseolus leaves have suggested that treatment with CCC may result in increased cell division in the leaves by controlling gibberellin liberation from the cotyledons. This would be an example of the cotyledons being directly involved in controlling cell divisions in the leaves as well as providing general substances such as carbon skeletons which are necessary for growth.

Fletcher, Peterson and Zalik (1965) showed that the differential effects of red and blue light on hypocotyl expansion of etiolated Phaseolus was lost on removal of the cotyledons; also suggesting a direct involvement of the cotyledons with the light stimulus.

The attempt to satisfy the requirement for the cotyledons by providing an external organic food supply was unsuccessful, indicating that some specific factors, which were not supplied in the medium, may be involved in the light response. However this is rather negative evidence, since it was not shown that substances in the medium were taken up by the plants. In work done with leaf discs (Liverman et al. 1955, Miller 1956, Powell and Griffith 1960 and Dale 1966), cell divisions were induced in leaf material in the presence of a carbon source and minerals, which suggests that the requirement for the cotyledons may be solely as a food supply. However there may be some difference in the behaviour of the leaves on the plant and that of discs cut from the leaves.
Biochemical investigations have shown that increase in levels of DNA, RNA and protein occurs in the leaves as a result of brief irradiation. It could be that, in the absence of the cotyledons, the response to light cannot occur because of the absence of carbon skeletons necessary for the manufacture of these substances. However, in the experiment in which plants without cotyledons were transferred to continuous light on day six, greening occurred and the leaves expanded. Mego and Jagendorf (1961) have shown that chloroplast development, on illumination of Black Valentine beans, involves a doubling of the protein content, while the lipid content shows a 50% increase. The observed chloroplast development in our experiments thus means that some food supply is available to the leaves in the absence of the cotyledons, and this, in turn, suggests that the cotyledons may be involved in the light response in some role other than merely to supply carbon skeletons to the leaves.

In the dark, between days four and six, although the protein content of the leaves is increasing, the protein per cell is decreasing. This is the period of active cell division in the leaves: thus we may conclude that, in the dark, increase in the protein content of the cell cannot keep pace with cell division. An increase in the protein content per cell was not observed until the rate of cell division slowed down and finally stopped. In plants exposed to white light for ten minutes however, increase in protein in the cell kept pace with the light
induced cell divisions and there was even an increase in protein per cell between days six and eight. Protein content of the cell depends on the rate of synthesis and also on the rate of breakdown of protein in the cells. Thus the increase in protein content of the cells, as a result of the light treatment, could be due either to an increase in the rate of synthesis or a decrease in the rate of breakdown of protein, or possibly to a combination of both. It is not possible, on the present evidence, to differentiate between the two; however, if increased rate of synthesis is involved, this could be due either to an increased supply of amino-acids, or to an increased ability of the leaf to use the available amino-acids.

Working with corn leaves, Mitrakos et al. (1965) demonstrated increased utilisation of soluble sugars by etiolated leaves which were irradiated briefly with red light. Thus it is possible that one of the actions of the light stimulus is to increase the ability of the leaves to use the materials available for growth.

Considered both on a per cell basis and on a per leaf basis, RNA content rose in the dark until cell division stopped on day eight. The result of brief light treatment was to increase RNA content per cell over the period during which cell divisions occurred in the leaves. A similar increase in RNA per leaf pair (approximately a doubling) was also observed in an experiment done in association with Dr. Loening of this department (unpublished), in which a different method of
extraction of RNA was used. This increase in RNA content of the leaves as a result of illumination is consistent with Mohr's (1966) suggestion that phytochrome might effect plant growth through gene activation. However, it is impossible to tell whether the RNA increase is a direct effect of the light treatment, or whether it is a secondary effect resulting from other physiological changes in the leaves initiated by the light treatment. It is probable that, as found by Weidner and Mohr (1967), the large changes in RNA content brought about by the light treatment are not the primary effects of the illumination.

The DNA content of the cells of the dark-grown leaves increased between days four and six, presumably due to an increasingly larger number of cells reaching the $4C$ condition prior to division. The fact that this high value was maintained between days six and eight, while the number of dividing cells is decreasing, may indicate that some of the cells stop dividing while they are in the $4C$ condition. If this is so, then it would appear that the cessation of cell division in dark-grown leaves between days six and eight is not due to failure of DNA replication in the dark, but rather to the absence of some other factor necessary for cell division which is formed as a result of illumination.

The drop in DNA content of the cells of dark-grown leaves which was observed to occur between days eight and thirteen has been mentioned earlier (page 69). This decrease could be due to death of cells in the leaf accompanied by breakdown of DNA, or
it could be due to cells which have reached the 4C condition, but which have not divided losing their newly replicated DNA. Habeshaw (1966) observed a gradual decline in the average DNA content of the nuclei in the outer cortex of ageing cultured pea root segments using Feulgen densitometry to determine DNA content. This indicates that a gradual loss of DNA from the nuclei is possible, but would appear to be associated with death of the cells. Whether cells could be reduced from the 4C to the 2C state by differential breakdown of DNA is a different matter, the main argument against it being that the enzyme system responsible for the breakdown might also affect DNA which is needed by the cell. However, since, in the cells, specific destruction of some proteins and RNA can occur, it is possible that specific breakdown of DNA might also occur.

Illumination of the leaves on day six resulted in a rapid decrease in the DNA content per cell. This rapid drop in DNA is what would be expected if there were cells in the 4C condition in the dark-grown leaves which divide on exposure to light, and thus substantiates the idea that on day six, in the dark, there are a large number of cells in the 4C condition. By day thirteen the DNA content per cell of the light treated leaves had dropped further, showing, as in the dark-grown leaves, a decrease in the mean DNA content per cell over a period when few cell divisions would be occurring, i.e. between days eight and
thirteen. A detailed examination of nuclei in the leaves using Feulgen densitometry might help to clarify the changes in DNA content observed in the dark grown and light-treated leaves. However a preliminary investigation using this technique showed that the material was not very suitable for this type of investigation. The small size of the cells made it difficult to cut sections one-layer thick and the close proximity of the nuclei, especially in the palisade, made measurements difficult.

It has been suggested that the factor which results in cell division stopping by day eight does not involve failure to replicate DNA by the nucleus. It is of interest that, although cell division ceases at the same age in light-grown leaves, the factor which brings about this must differ from that acting in the dark-grown leaves. In the dark-grown leaves further cell divisions can be induced by means of light treatment even on day ten, but, in light-grown leaves, divisions stop on day eight, even in the absence of light. It has been shown by Felippe (1967) that treatment of dark-grown plants with CCC leads to an increase in the cell number of the primary leaves, although it does not increase cell number of light-grown leaves. This is further evidence that the mechanisms which stop cell division in dark- and light-grown leaves are different. There is no evidence, however, as to how either mechanism works.

The decreasing response to light in terms of cell division, in leaves older than eight days, must also be considered. This decreasing response is common to both brief and prolonged light
treatments. The decrease in response occurs at the age at which cell division ceases in the dark-grown leaves, and it is possible that this coincidence is significant. Any one of three possibilities could explain the change in response of the older leaves. First the cells themselves could be losing their ability to divide; secondly, the materials necessary for cell division could become limiting, or, thirdly, the leaves could be losing their ability to receive the light stimulus. This situation parallels the decreased response of the cell expansion mechanism to light in the older leaves. The availability of materials, here necessary for cell division and the formation of new cells, and the decreasing food store in the cotyledons has been discussed when cell expansion was being considered. It is difficult to consider a possible loss of ability of the leaves to perceive the light stimulus since the method of action of phytochrome in bringing about cell division is unknown, as also is the system involved in the response of the leaves to prolonged illumination. However, since the two mechanisms are involved here, it is probable that, rather than the ability of the leaves to perceive both forms of stimulus being lost, it is the ability of the cells to respond to the stimulus which is lost. That is to say, either the cells are losing their ability to divide, or materials necessary for division are not available. This is further substantiated by work carried out by Felippe (1967), who has shown that the cell division response of the leaves to CCC is not found for older plants.
it is of interest that this decrease in response to light parallels the decrease in DNA content per cell; this, along with the close correlation between the decreasing response of both the cell division and the cell expansion mechanisms to light, suggests that major changes have taken place in the cells in the older leaves.

The main conclusions which have been drawn from this investigation can be summarised as follows:-

1. Light stimulates both cell division and cell expansion in the primary leaves of Phaseolus.

2. The degree of response of the cell division and the cell expansion mechanisms depends on the age of the plant at the time of treatment. Older plants respond less readily.

3. Cell division in dark-grown leaves can be stimulated by a brief illumination. This response is controlled by the phytochrome system.

4. Large increases in cell volume are not stimulated by this mechanism; prolonged illumination is necessary for marked increases in the mean cell volume of the leaves to occur, and red, blue and far-red light are all equally active in causing stimulation.

5. Additional cell divisions can be stimulated through a mechanism which involves prolonged exposure of the leaves to light. As with the response of cell expansion, red, blue and far-red radiation are equally
5. (contd) .... active in bringing about the cell division response.

6. The cotyledons must be present for the red, far-red phytochrome mediated reversible cell division response to occur. Presumably they are involved in supplying food substances to the leaves, but the results indicate that they may be involved directly in the perception of the stimulus, and in transmitting growth factors to the leaves.

7. The roots are also involved in the cell division response to light.
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