ANALYSIS OF GROWTH PATTERNS IN BARLEY COLEOPTILES

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By

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Barley coleoptiles were observed to be remarkably variable in height at 72 hr of germination. The objective of the study presented here was to analyze this variation in growth among coleoptiles and to attempt to determine its cause. The first step in this analysis of the variation was to determine the growth patterns of 900 individual coleoptiles by measuring their heights periodically during their growth period. In determining the cause for the variation, genetic, environmental, hormonal, metabolic and cytoplasmic factors were considered. For example, since gibberellin and kinetin have been implicated in the control of cell division and cell elongation these growth factors were supplied exogenously to germinating seedlings in an attempt to stimulate uniform growth of all coleoptiles. In similar studies, the effect of physical conditions and CO_2 on coleoptile growth was

determined. Variation in growth is shown by any one of a number of parameters. Proteins, however, because they are closer to gene activity than the others are a better indicator of whether the variation in growth is caused at a fundamental level. Variations similar to those in coleoptile height were found in the amino acid analysis of different types of coleoptiles. Experiments were also done to determine if a genetic component was responsible for the variation in coleoptile growth. There was no correlation between germination pattern of a seedling and that of its progeny; therefore, the variation in growth was attributed to differences in cytoplasmic constituents of individual coleoptiles.

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INTRODUCTION

1. Variation in Coleoptile Growth

Variation is an inherent part of all biological systems. Some variation is obvious, for example, variation seen among organisms, but even when it is not obvious variation is usually present. Even in inbred populations not all individuals are identical. A plant variety is produced by selection for a limited number of characters and though for these characters the population may show a high degree of uniformity it is rarely uniform for all characters. Variation that is not obvious viz. is hidden, can be particularly undesirable from an experimental point of view since it introduces an unexpected inconsistency in response to treatments. The ultimate source of variation must be genetic, although some variation could reflect physiological differences resulting from environmental flux. An example of this would be variation in growth rate due to differences in the microenvironments of seedlings.

An example of variation in growth is found in the coleoptiles. This variation had been observed in this laboratory on coleoptiles of barley. Heights in numerous

replicates, each of 200 seedlings, were consistently found to be highly variable. Subsequently it was shown that variation in coleoptile height is not confined to barley (Hordeum vulgare) but was also present in Zea mays, Triticum vulgare, Secale cereale, Avena sativa. Variation in seedling height was also found in Cucurbita maxima. In barley, variation is present in all seed samples, but the extent of variation differs in

1) different varieties
2) the same variety harvested in different years
3) the same variety grown in different locations

The variation in coleoptile height 72 hr after seeds were set to germinate was unexpected, particularly since coleoptiles have been so widely used in auxin bio-assays. Perhaps this observation should not have been unexpected. It is well known that the response of coleoptile segments to auxins is also variable (Went and Thimann, 1937). Bearing these observations in mind, it is difficult to decide whether in fact coleoptiles are a suitable test system for experimental purposes. Without experimental analysis one cannot relate variation in growth rate and sensitivity to experimental treatment in any meaningful way.

It was clear therefore that any further use of coleoptiles as a test system for bio-assays must be preceded by 1) an analysis of the growth pattern of large groups of coleoptiles and 2) a definition of the limits of

the variation in growth. The pattern of growth of barley coleoptiles has been determined and forms the basis for the studies presented here.

Went and Thimann (1937) reported that growth rates of Avena coleoptiles increased from time of sowing and reached a maximum of 1,000 μ/hr by 72 hr. Each sample measured consisted of 30-50 coleoptiles. This result suggests that growth of these coleoptiles is uniform. Auxin can stimulate 1) elongation of coleoptile segments when they are placed in auxin solutions or 2) coleoptile curvature upon application of auxin on one side of a decapitated coleoptile. Both of these tests have been used as bioassays for auxin, however, Avena coleoptiles have always shown marked variation in response to auxin. To overcome this variation some workers selected coleoptiles on the basis of height (McCrae and Bonner, 1953; Nitsch and Nitsch, 1956). Even with selected coleoptiles recent reports still indicate that "certain effects of auxin cannot be demonstrated in every trial." (Rayle et al., 1970). Thus in spite of using a test system that is morphologically uniform, the variation in response to auxin remains. The relationship, therefore, between the heights of coleoptiles and their individual growth rates should be considered.

Lack of uniformity in growth rate of coletoptiles must reflect variation in physiological processes. Respiration rates, for example, vary from seedling to seedling.

Stiles and Leach (1932) determined respiration rates of individual sweet pea seedlings and showed that rates increase rapidly early in germination in some seedlings, while in others rates remain low for a time before increasing to a high level. These two types of seedlings showed a rapid growth rate; other seedlings grew slowly and had a respiration rate that remained at a low level. Comparison of these results with those from auxin bioassays suggest that it may be more valid to select coleoptiles on the basis of both growth rate and height and this implies selecting them for high physiological activity, rather than selecting them only on the basis of height.

The first step in this study of coleoptiles was to quantitate the variation in their growth rate and to determine its effects on their response to auxin. One advantage of using barley coleoptiles is that their relatively short growth period permits rapid analysis. The second advantage is that those aspects of the germination of barley relating to endosperm reserve degradation have been extensively studied (Varner and Johri, 1968). Results of these studies provide a useful body of data on the availability of food reserves at different times during germination.

2. Effects of the Physical Environment on Growth

Even small changes in the physical environment can affect plant growth. For example, plants, unlike animals, are not homotherms and slight changes in temperature can alter their growth rate significantly. Thus maintenance of physical conditions at constant levels would appear to be necessary for reproducibility of results. Yet with water levels, many workers do not specify how much water is used for seed germination. The only indication given in some cases is that seeds were placed on moistened filter paper or sand. It is likely that experimental results may vary as much from differences in the volume of water available as from the treatment. For the results reported here the optimum physical conditions for barley coleoptile growth were determined and used in all experiments unless otherwise stated i.e. seeds were germinated on filter paper in dishes containing 12 ml of water and grown at 25 C in the dark.

3. Coleoptile Growth Patterns

The heights of coleoptiles grown under standard conditions are remarkably variable at 72 hr; they do not form a normal distribution. Furthermore, the form of the

distribution of heights changes during germination. It is a normal distribution only early in germination and then spreads rapidly until between 66-70 hr it is trimodal. The trimodal distribution changes very quickly to an almost normal distribution skewed to the shorter coleoptiles. The changes in the form of coleoptile height distribution reflect the following:

1) variation in the rates of elongation of each coleoptile
2) differences in the time and duration of maximum growth

Six growth patterns are evident as a result of the variation in time and rate of growth of individual coleoptiles. These are:

I: rapid elongation begins early, at 34 hr, lasts about 40 hr, tall final height.

IIa: active elongation begins early, lasts about 40 hr, growth rate is less than I, medium final height.

IIb: active elongation begins slightly later, lasts about 50 hr, tall final height.

IIIa: elongation is slowest of all the classes, lasts about 70 hr, short final height.

IIIb: active elongation begins late, at 70 hr, lasts about 40 hr, rate is similar to IIa, medium

final height.

IIIc: rapid elongation begins late, at 70 hr, lasts about 40 hr, rate is similar to I, tall final height.

These results show that different coleoptiles grow at different rates and at different times. They show that a sample of coleoptiles taken at random is made up of individuals with different growth patterns. This analysis makes it possible to select coleoptiles that are similar both in height and growth rate. This cannot, however, be done at all times during germination. Class I coleoptiles, for example, are not recognized accurately until about 44 hr of germination. The other major classes, II and III, can be selected at 66 hr; their subclasses do not become easily distinguishable until 76 hr.

4. Growth Factors

Since the initiation of rapid growth does not occur simultaneously in all coleoptiles it was concluded that coleoptiles that germinate slowly may have only a limited supply of some necessary factor. This factor may be required for cell division or elongation or both. Cell division and elongation both contribute to coleoptile growth (Wright, 1961; Rose and Adamson, 1969). Kinetin has been reported to

initiate cell division (Guttman, 1956) and gibberellin to stimulate cell elongation (Lockhart, 1965). Both these growth factors stimulate elongation of excised wheat coleoptiles (Wright, 1961). Thus, if the factor in limited supply in slowly growing coleoptiles is a kinetin or gibberellin-like substance, exogenous application of these compounds should overcome the internal deficiency; they should produce, in the whole population, a uniform rate of growth early in germination.

None of the growth factors used induced a uniform rate of growth early in germination. Gibberellic acid and kinetin inhibited growth when given in the first 24 hr of germination. Therefore, it seems that internal deficiencies in slowly growing seedlings cannot be corrected by exogenous treatments with growth factors.

5. Protein Analysis

In germinating wheat, protein synthesis is initiated soon after soaking and increases rapidly after 15 hr (Marcus et al., 1966). Also, at 15 hr, new species of messenger RNA and transfer RNA begin to be synthesized (Vold and Sypherd, 1968). If barley behaves in a similar way then coleoptiles that initiate growth early may be synthesizing new and different types of proteins. Failure to initiate early rapid

growth may reflect a delay in the onset of protein synthesis; indeed, class II and III coleoptiles may be delayed in growth as a result of a delay in the synthesis of proteins necessary for rapid growth. A simple way of determining gross changes in levels and composition of proteins is by a total amino acid analysis. The protein levels do increase and composition changes in coleoptiles that initiate an active growth early in germination (Class I). These changes also take place but at later times in coleoptiles that do not initiate an early rapid growth rate (Class II, III). Initiation of active growth thus requires synthesis of new proteins. The results do not indicate, however, what factors cause the delay in protein synthesis in slowly growing coleoptiles.

6. CO_2 Effects on Coleoptile Growth

CO_2 has recently been shown to stimulate elongation of coleoptile segments (Rayle and Cleland, 1970), though in other organs, roots and shoots, it inhibits growth (Mer, 1957; Stoljwik and Thimann, 1957; Geisler, 1963; Geisler, 1967). CO_2 also inhibits respiration (Beevers, 1961). Respiration is an important process in seed germination. The difference in the reported responses of coleoptiles and shoots to CO_2 suggested a relationship during early germination. CO_2 respired by the plumule enters the chamber

formed by the coleoptile; a high level of CO_2 might, at the same time, inhibit plumule growth and stimulate coleoptile growth. This situation would continue while the seedling is under the soil or in a dark chamber. Once the shoot is in the light, however, the photosynthetic machinery of the plumule develops rapidly and some of the respired CO_2 will be assimilated. Release of CO_2 into the coleoptile chamber will be reduced. Though this may not be due solely to an effect of CO_2 on relative rates of growth of the plumule and coleoptile, once seedlings are illuminated, coleoptiles stop growing rapidly.

It appeared that there might be a relationship between coleoptile growth and internal CO_2 levels. Whole seedlings and coleoptile segments were treated with carbonate solutions and phosphate buffer of similar pH. The results suggested that CO_2 levels do modify relative growth rates of plumules and coleoptiles. Thus, though CO_2 respired by the plumule may affect coleoptile elongation it is clear that CO_2 alone is not the agent controlling the growth pattern of class I, II and III coleoptiles. Since plumule growth is also slow in seedlings with class III coleoptiles it is clear that there is an overall control of growth that operates in plumules and coleoptiles. Once this has been established it is modulated only slightly by CO_2.

Coleoptiles of germinating barley do not grow uni-

formly. This non-uniformity reflects coleoptiles in different stages of development. Since some coleoptiles begin active growth sooner than others it appears they have sufficient internal stimulus for an early rapid growth. This stimulus is probably deficient in coleoptiles that grow slowly for a long time before initiating rapid growth. In the studies presented here, an analysis of the growth of individual coleoptiles is done. The differences in growth of coleoptiles are related to variation in response of coleoptiles to auxin. Causes for the lack of stimulus in some coleoptiles to initiate active growth early in germination are also considered.

METHODS AND MATERIALS

1. Germination Conditions

Foundation stock barley seeds (*Hordeum vulgare*, variety Brock) untreated with fungicides and pesticides, were germinated on two 12.5 cm circles of Whatman No. 1 filter paper in 15 X 2 cm petri dishes. There were 30 seeds per dish. Distilled water was used to moisten the paper and the dishes were then covered and kept in darkness in incubators at 25 C. Individual coleoptile heights were measured periodically under weak red light between 21-100 hr after the beginning of soaking.

In the initial experiments 300 seeds were used and the effects of water, temperature (15, 19 and 25 C) and light on coleoptile growth were determined. Ten, 12, 15, or 25 ml distilled water were used per dish. With ten ml per dish some drying out occurred over 100 hr and the amount of water used subsequently was therefore 12 ml. Height, fresh weight and dry weight of uniform class I coleoptiles grown at 25 C were determined at 15, 22, 33, 42, 68 and 115 hr of germination.

2. IAA Treatments of Coleoptile Segments

For treatments with IAA, coleoptile segments were floated, in 4.5 X 1 cm petri dishes, on ten ml of either distilled water or 10^{-5} M IAA at 25 C for three hr. Thirty segments were used for each test. One five mm segment was cut from each coleoptile four mm below the apex. Segments were taken from three classes of coleoptiles: a) 15-20 mm high at 48 hr, b) 21-26 mm high at 70 hr and c) 15-20 mm at 75 hr. Seedlings were grown in the dark and segments were handled under dim red light. Coleoptile segments were measured at the end of the three hr period.

3. Coleoptile Cell Number

At 12, 24, 34, 44, 68, 78 or 115 hr from the beginning of germination the cells in a vertical column were counted. Four columns of parenchyma were counted in each of five coleoptiles. Cell length was calculated from cell number and coleoptile length.

Dry barley seeds were irradiated using a ^{60}Co source. The dose was 250 Krads delivered in 25 min. Seeds were germinated immediately after irradiation. Coleoptile heights were recorded intermittently during germination up to 119 hr. At 119 hr, cell numbers were determined, as above, on coleop-

tiles 6, 19, 23 and 32 mm in height. Five coleoptiles were used for each height.

4. Amino Acid Analysis

At 12, 18, 24, 34, 44, 68, and 85 hr of germination, class I coleoptiles and at 85 hr class IIIa, IIIb and IIIc were selected for amino acid analysis. Ten coleoptiles from each class at the indicated ages were measured, weighed and then macerated in 80 % ethanol with a pestle and mortar. After centrifuging, the alcohol soluble fraction was dried and then passed over a column of Dowex 50 X 8 cation exchange resin. The resin was washed with water and the amino acids were then eluted with 2N HCl, dried and made up to volume in water. The water used during all operations was glass distilled. Amino acid analysis was then performed on the sample with a model 120 C Beckman automatic amino acid analyzer.

The alcohol insoluble fraction was hydrolized in 6N HCl for 12 hr at 100 C. The HCl was then removed by evaporation and the hydrolysate was treated in the same way as the alcohol soluble fraction. A triplicate run was done on the 44 hr class I coleoptiles.

5. Kinetin and Gibberellin Treatments

In the first treatment 600 seeds were germinated and grown in dishes containing either 10^{-5} M gibberellin A_3 or 10^{-5} M kinetin (6-furfurylamino purine). These were continuous treatments for 66 hr. Coleoptile heights were measured at 66 hr. For the second treatment 150 seedlings, each having three or more roots emerged, were selected after 24 hr germination on distilled water. These seedlings were transferred from dishes containing water only to petri dishes containing 12 ml of either 10^{-5} M gibberellin or 3 X 10^{-6} M kinetin for one of the following times: 24-68 hr, 24-34 hr, 34-44 hr, 34-68 hr, 44-54 hr, 44-68 hr. After treatment, the roots were rinsed in distilled water to remove traces of the growth factor. The seedlings were returned to dishes containing distilled water. Coleoptile heights were measured at 68 hr.

In the third set of treatments 100 seedlings were selected 24 hr after the beginning of germination; only the coleorhiza had emerged from these seedlings. They were treated from 24-34 hr with a 10^{-4} or 10^{-5} M solution of gibberellin, then washed in distilled water and grown until 68 hr. At 68 hr coleoptile heights were measured, fresh weights of coleoptiles and plumules combined were obtained and after drying in an oven at 100 C, dry weights were determined.

Untreated seedlings were grown in dishes that contained only distilled water.

6. Inositol and IAA Treatments

One hundred and fifty seedlings that had three or more roots at 24 hr were selected and transferred to dishes containing myo-inositol. In this thesis inositol always refers to myo-inositol. Seedlings were treated from 24-68 hr at three concentrations: 10^{-2}, 10^{-4}, or 10^{-6} M. At 68 hr coleoptile heights were measured and fresh and dry weights of the roots and shoots were determined. In the second experiment 150 unselected seeds were germinated for 82 hr on distilled water, 10^{-5} M IAA of 3 X 10^{-6} M inositol. At this time coleoptile heights were measured.

7. Carbon Dioxide Effects on Time of Plumule Emergence Through the Coleoptile

A relatively uniform population of seedlings, early rapid growth rate, were selected at 48 hr. Sixty seeds per treatment, 30 per 15 X 2 cm petri dish, were grown under light or dark conditions at 25 C until 96 hr of germination in 12 ml of 1) distilled water 2) 0.1 M $NaCO_3$, pH 8.0 3) 0.1 M $NaHCO_3$ adjusted to pH 6.0 with HCl or 4) 0.1 M phosphate

buffer, pH 6.0. The time of plumule emergence from the coleoptile chamber was recorded for each seedling. Heights of the coleoptile and plumule were noted at the time of plumule emergence and at 96 hr.

8. CO_2 Effect on Coleoptile and Plumule Segment Elongation

Coleoptile and plumule segments, 0.5 mm long and taken four mm below the apex, from three different types of seedlings were incubated for three hr in 1) distilled water 2) 0.1 M $NaHCO_3$, pH 8.0 3) distilled water that had CO_2 bubbled into it for two hr prior to the experiment or 4) 0.1 M phosphate buffer, pH 6.0. The three types of selected seedlings were a) 11-16 mm at 48 hr, 60 segments b) 26-30 mm at 72 hr, 60 segments and c) 40-60 mm, 30 segments at 96 hr. Plumules had not emerged from any of the coleoptiles used. All experimental manipulations were done under a weak red light.

9. Stomata Number

The number of stomata on the inner epidermis of ten class I coleoptiles was recorded at 15, 46 and 68 hr of germination. Determinations were also made for slowly growing class III coleoptiles at 68 hr and the subclasses IIIa, IIIb

and IIIc at 75 and 115 hr of germination.

10. Effect of Seed Coat and CO_2 on Coleoptile Growth

Seedlings were germinated for 24 hr and then those that had only the coleorhiza, no roots, emerged were selected for the experiment. Half of the seedlings were decoated and half were left with their coats intact. Seedlings, 150 per treatment, were then put in either 1) distilled water or 2) 0.1 M $NaHCO_3$, pH 8.0 in the dark until 68 hr. At 68 hr individual coleoptile heights were measured.

11. Inheritance of Coleoptile Growth Patterns

One class I seedling was grown to maturity; the seeds were collected, germinated, classified and also grown to maturity. The seedling germination pattern of the third generation seeds was compared to that of a random sample of seeds.

Final heights of 78 class I seedlings were also compared to the average final heights of 12 class IIIa seedlings.

RESULTS

1. Effect of Water Volume on Growth

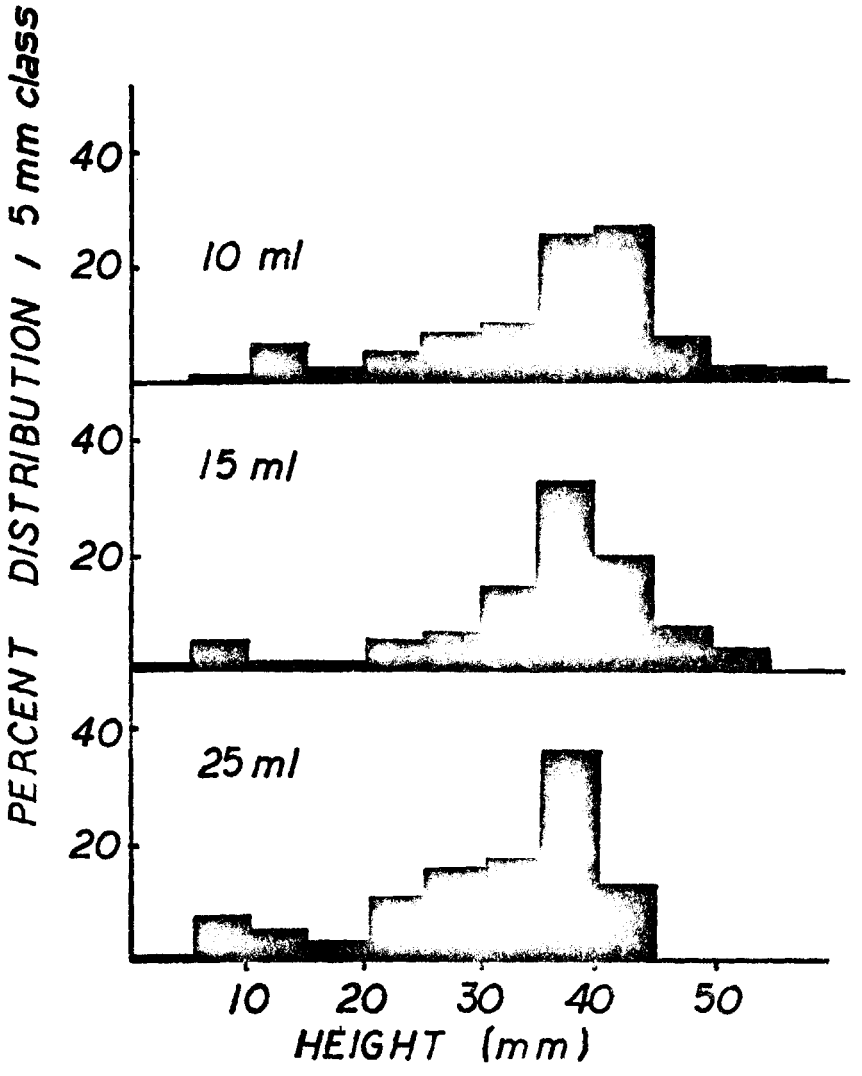
Defining suitable physical conditions for seed germination may seem unnecessary in view of the data already available (Mayer and Poljakoff-Mayber, 1963). It became obvious, however, that even slight changes in environmental conditions altered the growth pattern of coleoptiles. For example, small variations in the volume of water used for seed germination were found to modify coleoptile growth. The first step, therefore, was to find a set of environmental conditions under which there was good growth. It was not determined whether the conditions used were absolutely optimal. That would have required a more extensive analysis than seemed justified.

Barley was sown in dishes containing 10, 15 or 25 ml distilled water and coleoptile heights were measured 72 hr later. As the volume of water per dish increases, the frequency distribution of coleoptile heights changes. The frequency of tall coleoptiles decreases (Fig. 1). With 25 ml distilled water no coleoptiles reach 45-60 mm by 72 hr, cf. ten and 15 ml (Fig. 1). These results show that significant effects on coleoptile heights occur with small changes in

FIGURE 1

Effect of Water on Barley Coleoptile Growth

Frequency histogram of coleoptile heights of seeds grown in 10, 15 or 25 ml distilled water in the dark at 25 C. Three hundred seeds were used for each treatment. Heights were measured at 72 hr and grouped into five mm classes. The numbers of coleoptiles on which each histogram is based are 219 (10 ml), 158 (15 ml), 128 (25 ml).


PERCENT DISTRIBUTION / 5 mm class
40
20
10 ml
40
20
15 ml
40
20
25 ml
10
20
30
40
50
HEIGHT (mm)

water volume. The effect of water volume is emphasized since 1) the volume is often not specified by some investigators and 2) 15 and 25 ml of water retard the growth of those coleoptiles that elongate most rapidly in the first 72 hr of germination. The effect is to produce a cluster of heights in the 31-45 mm range. This reduced range of coleoptile heights gives the appearance of increased uniformity but actually hides a potential for variability. This effect could be important since the volume of solution used could affect growth as much as an experimental treatment. Because drying out occurred with ten ml water and 15 ml retarded coleoptile growth, 12 ml of water per dish was used in subsequent experiments.

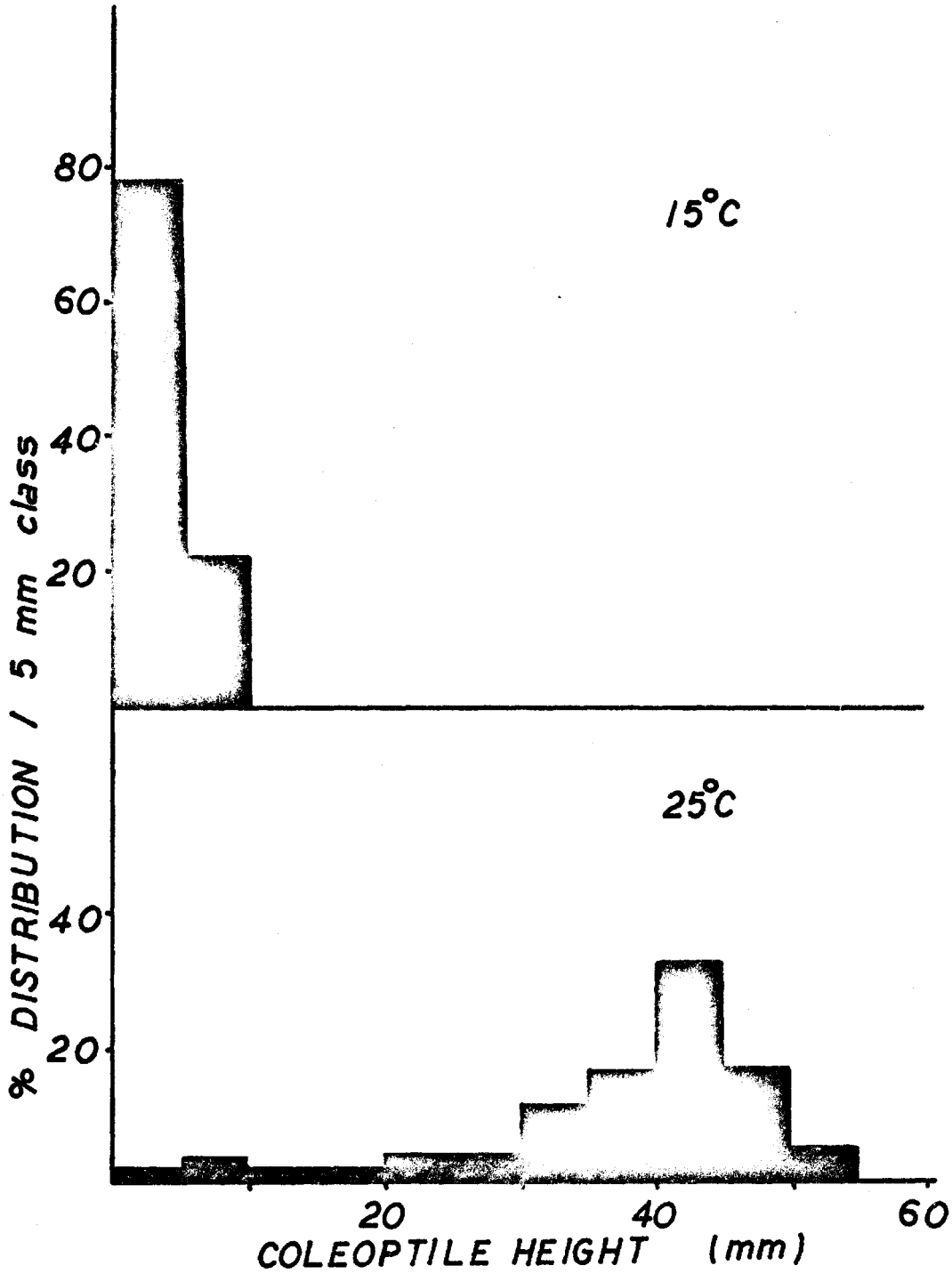
2. Effect of Temperature on Coleoptile Growth

Growth rates of seedlings are affected by temperature. When grown at 15 C the tallest coleoptiles are only 10 mm, while at 25 C, they are 55 mm by 72 hr of germination (Fig. 2). Neither final height nor the variation in coleoptile height is reduced by growing seedlings at 15 C rather than 25 C. Growth patterns are not changed at 15 C; coleoptiles merely grow at a slower rate than at 25 C. For example, the coleoptile height distribution at 72 hr of seedlings grown at 25 C is not seen until six days if they are grown

FIGURE 2

Effect of Temperature on Coleoptile Growth

Frequency histograms of coleoptile heights, five mm classes, of seedlings grown in the dark at 15 C or 25 C till 72 hr of germination. Three hundred seeds were used for each treatment and at 72 hr 234 had germinated at 15 C and 198 at 25 C.


80
60
40
20
15°C
25°C
40
20
% DISTRIBUTION / 5 mm class
20
40
60
COLEOPTILE HEIGHT (mm)

at 15 C. Since seedlings grow quickly at 25 C this temperature was used for germination in all subsequent experiments.

3. Light

In the initial experiments on etiolated coleoptiles, measurements were recorded only once during the germination period, e.g. at 72 hr. Seedlings were exposed to light, therefore, only at harvest time. In later experiments on growth rate, measurements were taken intermittently during the coleoptile growth period. Though these determinations were done in a dimly-lit lab, it was found that even these short exposures to light, accumulated over time, affected coleoptile elongation (Fig. 3). As the amount of light given to the coleoptiles increased from 6-40 min, coleoptile growth was reduced.

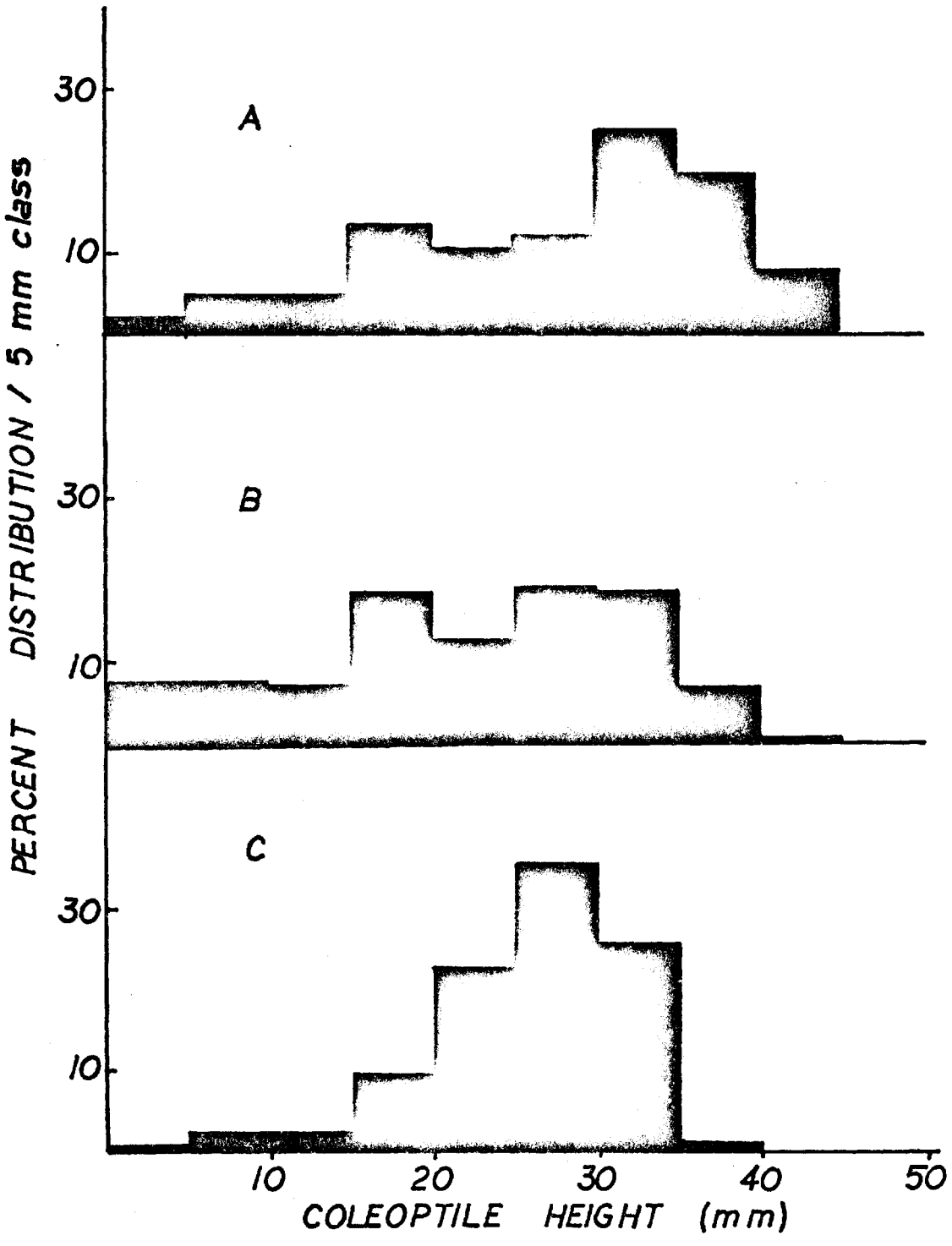
A total of 40 min of dim and 25 sec microscope light seemed to affect the coleoptile height distribution in two ways. After a total of 30 min of light, the percent of tall coleoptiles (35-45 mm) was greatly reduced. In addition, however, the height distribution pattern was different from the pattern after either six or 30 min of light because the shorter coleoptiles were stimulated to grow.

It is well known that light inhibits growth of etiolated coleoptiles (Mayer and Poljkoff-Mayber, 1963). The

FIGURE 3

Effect of Light on Coleoptile Elongation

Seedlings were exposed to a total of A: 6, B: 30 or C: 40 minutes of dim room light (5 fc). Seedlings in C also received about 25 sec of bright microscope light (30,000 fc). All light was received intermittently during measurements of coleoptile heights. Three hundred seeds were set out in A and B; 900 in C. Measurements were made at 66 hr of germination.


PERCENT DISTRIBUTION / 5 mm class
30
10
A
30
10
B
30
10
C
10
20
30
40
50
COLEOPTILE HEIGHT (mm)

interesting result from these short exposures to light is that they reveal that the response of all coleoptiles to light is not identical i.e. growth of tall coleoptiles is inhibited while short coleoptiles are stimulated. As with the water treatments the light effects indicated the presence of physiological variability in populations of coleoptiles. The significance of this was revealed by the study of growth patterns (section four). Since some light is necessary while coleoptiles are being measured, a source of dim red light which does not affect growth was used. The distribution of coleoptile heights at 68 hr was similar whether seedlings remained in the dark continuously or whether they were exposed to dim red light during the time when measurements were taken (1 fc).

4. Coleoptile Growth Patterns

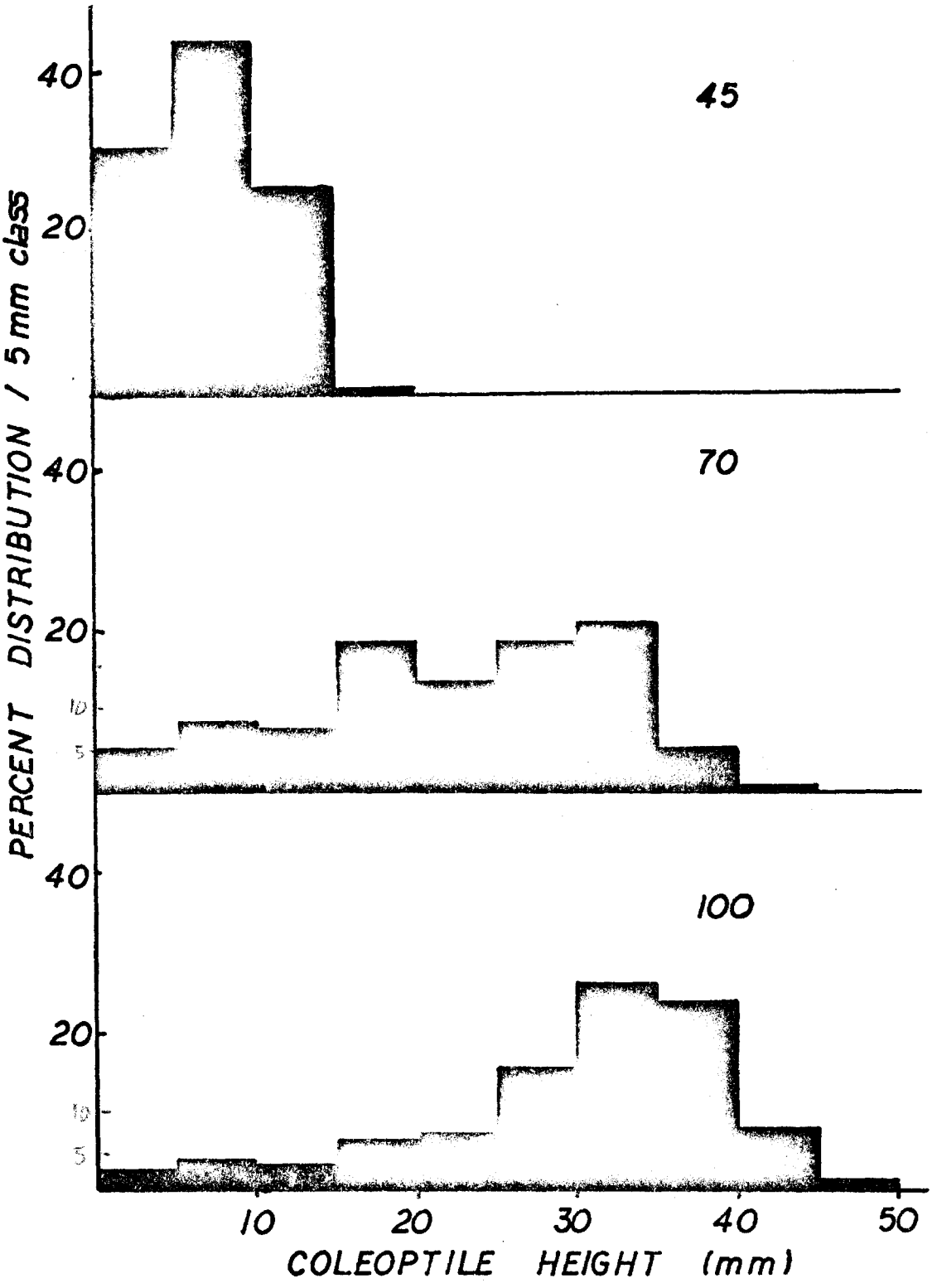
From these initial results (Fig. 1, 2) it is evident that all coleoptiles do not elongate uniformly. To put all the individual growth patterns found in a population of coleoptiles on a firm quantitative basis, elongation of a large number of individual coleoptiles was measured.

Frequency histograms of coleoptile heights change during the first 100 hr of growth (Fig. 4). At 45 hr all coleoptiles are short and the distribution of heights is

FIGURE 4

Change in Coleoptile Height Distributions During Germination

Frequency histogram of coleoptile heights at 45, 70 and 100 hr after the beginning of germination. Each dish contained 12 ml distilled water. The numbers of seedlings measured were 808 (45 hr), 819 (70 hr) and 827 (100 hr); 900 seeds were set out at a density of 30 seeds per dish.


45
70
100
40
20
10
5
PERCENT DISTRIBUTION / 5 mm class
10
20
30
40
50
COLEOPTILE HEIGHT (mm)

relatively normal. A different pattern is found between 66 and 70 hr. In this four hr period the distribution of coleoptile heights is trimodal; they appear to be grouped about three heights: long (31-35 mm), medium (16-20 mm), and short (6-10 mm). This distribution lasts only 4-5 hr and as coleoptiles grow, their heights revert to a more normal distribution slightly skewed to shorter height values. From 70-100 hr the extent of the skewness is reduced as the shorter coleoptiles increase in height. At about 100 hr growth stops; coleoptiles have then reached their maximum height and each is pierced at its tip by the emerging leaf.

The change with time in the frequency distribution of coleoptile heights shows that individual coleoptiles grow at different rates and times after the beginning of germination. A trimodal distribution of coleoptile heights is present at 66-70 hr and then disappears very quickly. This suggests that significant changes in growth rate of coleoptiles are taking place at this time. To show that coleoptile growth rates do change during germination, the growth rates of individual coleoptiles were determined.

Heights of individual coleoptiles were recorded over the whole growth period. Analysis of their growth reveals the extent of differences between coleoptiles. Three five mm classes taken from the peaks of the trimodal distribution were selected and classified as class I (31-35 mm), class II (16-20 mm) and class III (6-10 mm), (Fig. 4). The

final heights at 100 hr of coleoptiles of these classes are as follows: class I, 31-50 mm; class II, 16-45 mm; class III, 6-40 mm,(Fig. 5).

The class I coleoptiles measured at 70 hr (Fig. 4,5) show only a slight increase in length in the following 30 hr; 32 percent remained in the 31-35 mm height class, 52 percent moved up into the 36-40 mm height class and one percent reached 46-50 mm (Fig. 5). Class II and III coleoptiles however, increased significantly in height (Fig. 5, 6). In class II, for example, approximately 40 percent doubled in height. Thus, by 100 hr class II coleoptiles have subdivided into two groups: IIa coleoptiles which reach a final height of 16-25 mm and IIb coleoptiles whose final heights are 26-40 mm.

In class III an even greater range of final heights occurs (Fig. 5,6). Class IIIa coleoptiles are 6-15 mm in height; class IIIb 16-25 mm and IIIc coleoptiles are 26-40 mm high.

The three subclasses chosen from the frequency distribution in the period between 66 and 70 hr have resolved themselves into six subclasses. Each subclass has its own pattern of growth (Fig. 6,7), i.e. class I coleoptiles grow most rapidly between 45 and 60 hr and have almost reached their maximum height by 77 hr, while class II coleoptiles begin active growth somewhat later. In class IIa maximum

FIGURE 5

Final Height Distributions of Class I, II and III Coleoptiles

Classes I, II and III were selected from the three peaks of the trimodal height distributions at 70 hr (Fig. 4). Coleoptile heights of the three classes at 100 hr are plotted.

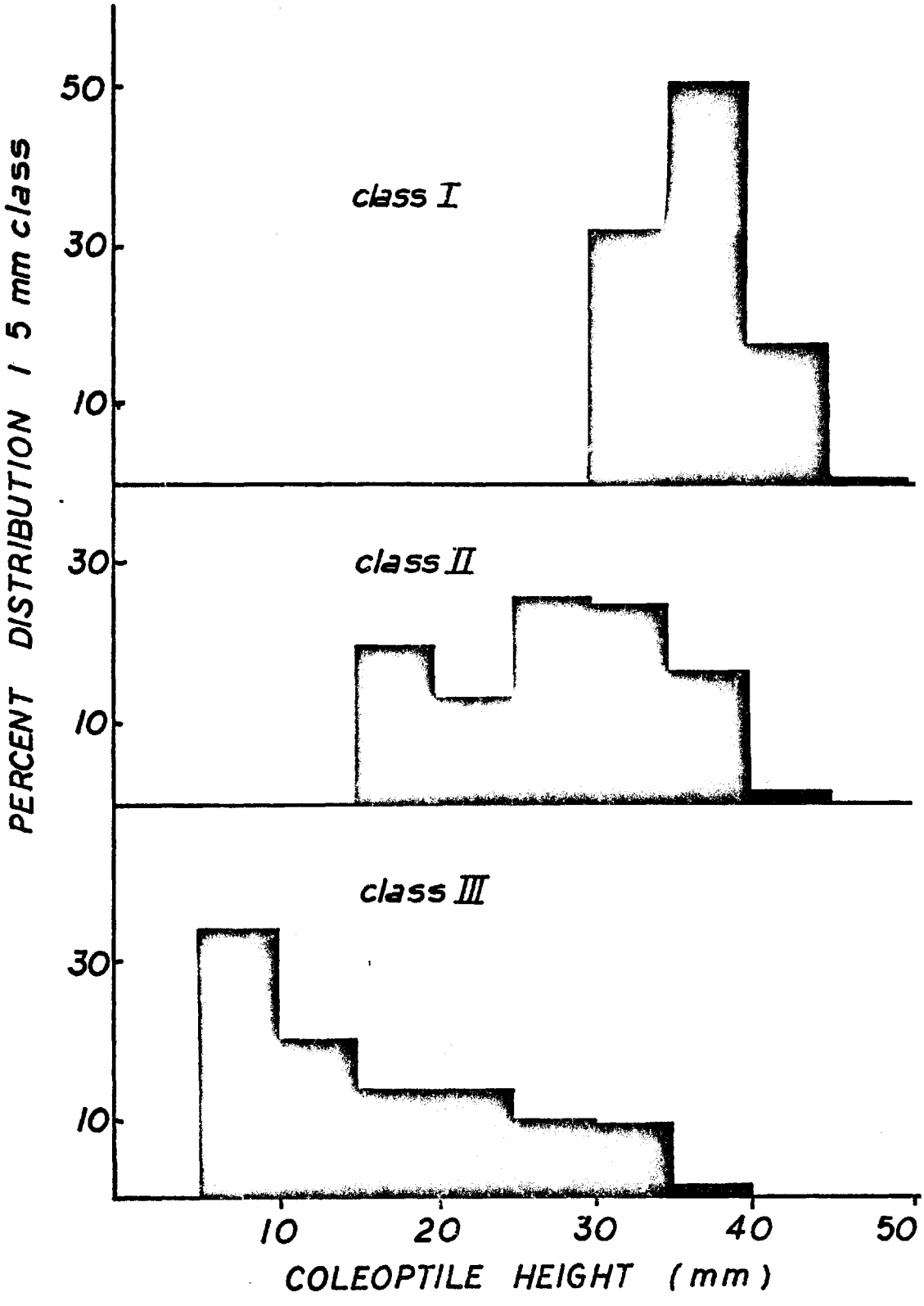

PERCENT DISTRIBUTION / 5 mm class
50
30
10
class I
30
10
class II
30
10
class III
10
20
30
40
50
COLEOPTILE HEIGHT (mm)

FIGURE 6

Individual Coleoptile Growth Patterns

Increase in coleoptile height from 21-100 hr from the beginning of germination. Heights of all coleoptiles were measured; those whose heights are plotted here were selected at 66 hr as class I (31-35 mm), class II (16-20mm) and class III (6-10 mm). Class I ●—● , class IIa ■····■ , class IIb □····□ , class IIIa ▲ ——··▲ , class IIIb ▶—··▶ , class IIIc △ —··△ .

The mean value and 2X the SD given for each coleoptile class are based on 168 (class I), 48 (class IIa), 101(class IIb), 12 (class IIIa), 18 (class IIIb) and 35 (class IIIc) coleoptiles.

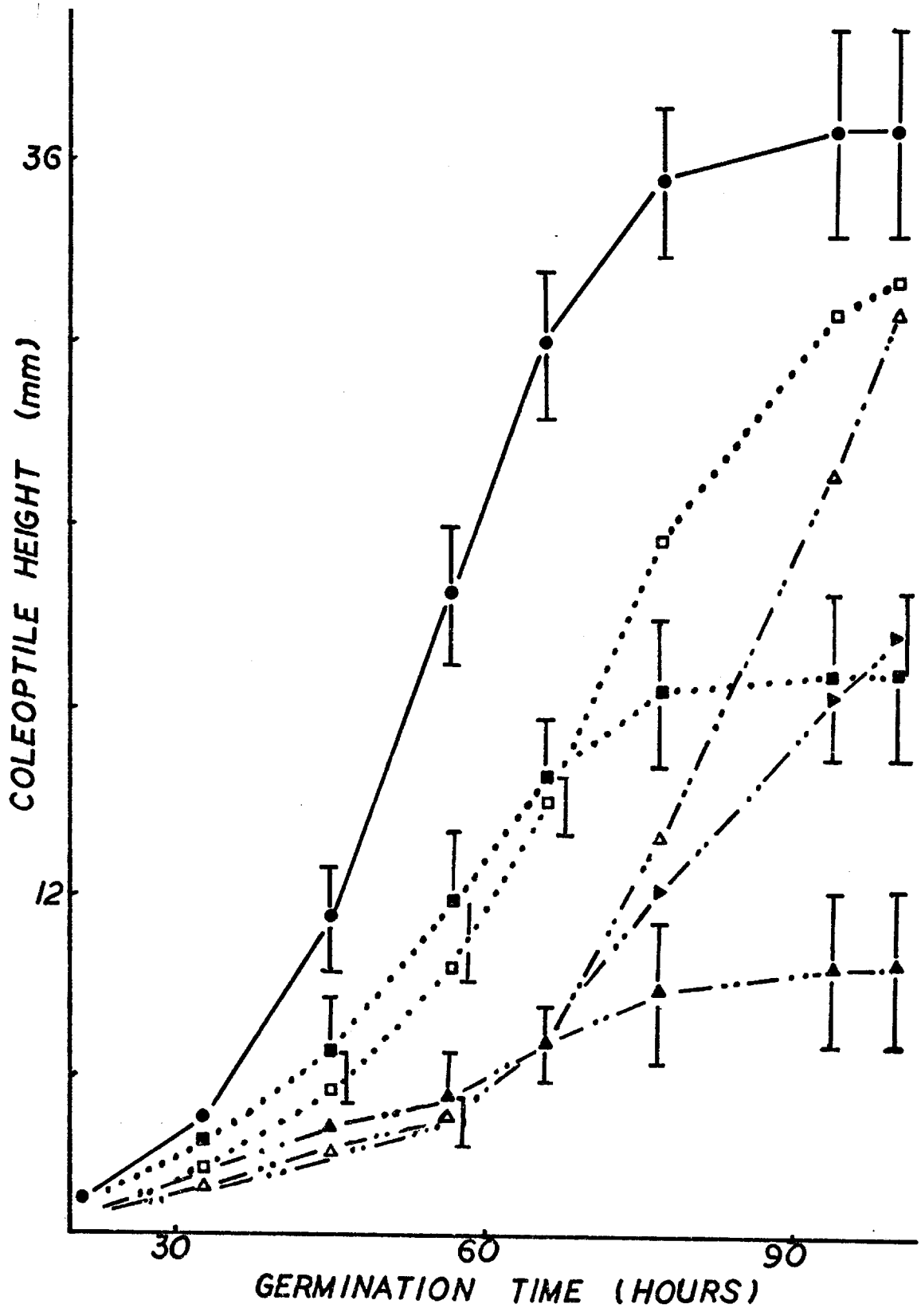
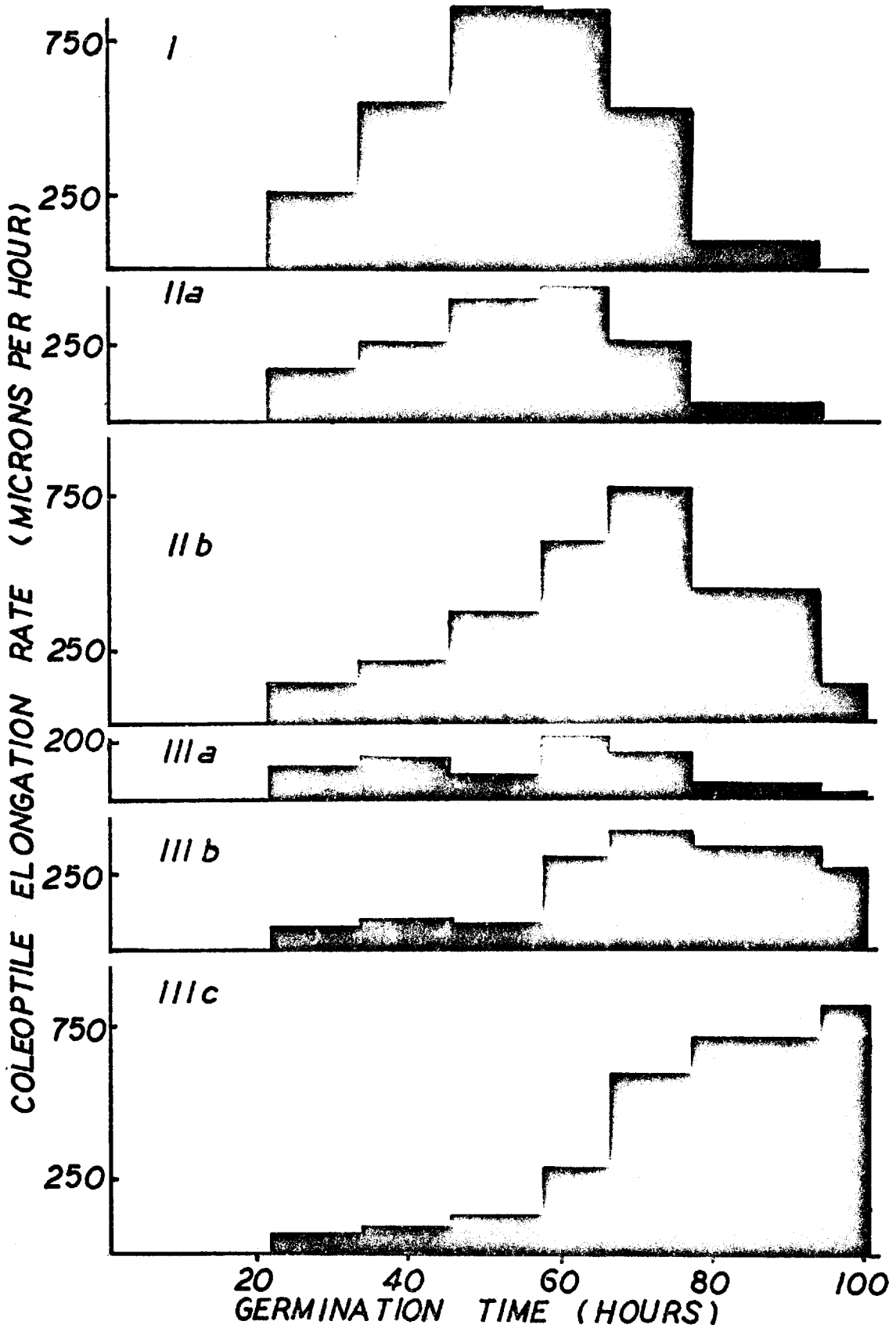

36
12
COLEOPTILE HEIGHT (mm)
30
60
90
GERMINATION TIME (HOURS)

FIGURE 7

Elongation Rates of Various Coleoptile Classes

Mean rates of elongation, μ/hr, of various classes of coleoptiles up to 100 hr from the beginning of germination. These values are from the same coleoptiles in Figure 6.


I
IIa
IIb
IIIa
IIIb
IIIc
750
250
200
COLEOPTILE ELONGATION RATE (MICRONS PER HOUR)
20
40
60
80
100
GERMINATION TIME (HOURS)

rate of elongation occurs about 60 hr but is relatively low, approximately 440 μ/hr in comparison with the maximum rates of elongation in coleoptiles in class I, 870 μ/hr or IIb, 770 μ/hr (Fig. 7). Class IIb coleoptiles achieve their maximum rate of elongation about 70 hr but they continue to elongate rapidly and by 94 hr are almost as tall as class I coleoptiles.

Class III coleoptiles are all very short until about 66 hr (Fig. 6) and then they separate into three subclasses. Class IIIa has a maximum elongation rate of 200 μ/hr and the maximum height they reach is about 10 mm. Class IIIb show an increased rate of elongation beginning at 66 hr and continuing to 90 hr. Their final heights are similar to those of class IIa coleoptiles. Class IIIc coleoptiles do not begin to elongate rapidly until about 66 hr also. Their rate of elongation, however, 800 μ/hr, is almost equal to that of class I coleoptiles. The crucial difference is that maximum growth in class IIIc and class I coleoptiles occur 50 hr apart.

The growth pattern of coleoptiles in any size class at 66-70 hr (Fig. 4) is similar to the pattern in the size class immediately adjacent to it, for example, coleoptiles whose heights are 21-25 mm at 70 hr form the size class adjacent to class II (Fig. 4). By 100 hr the heights of these coleoptiles range from 21-50 mm and their growth rates vary

in a manner similar to those of class II coleoptiles. Some show little growth after 70 hr while others grow rapidly between 70-100 hr. Thus, the growth patterns of the coleoptiles designated as class I, II or III are typical of the whole population of the coleoptiles.

The coleoptile growth patterns just described are not restricted to one temperature. Barley grown at 19 C also shows a trimodal distribution of coleoptile heights but while it is present at 66-70 hr at 25 C (Fig. 4) it is not seen until 96 hr at 19 C (Fig. 8). The three peaks of coleoptile heights in the ranges 11-15 mm, 21-25 mm and 36-40 mm at 19 C correspond to the three peaks seen at 25 C at 66-70 hr. Similarly the height distributions of 45 and 100 hr at 25 C (Fig. 4) are seen at later times i.e. 60 and 140 hr respectively when seedlings are grown at 19 C.

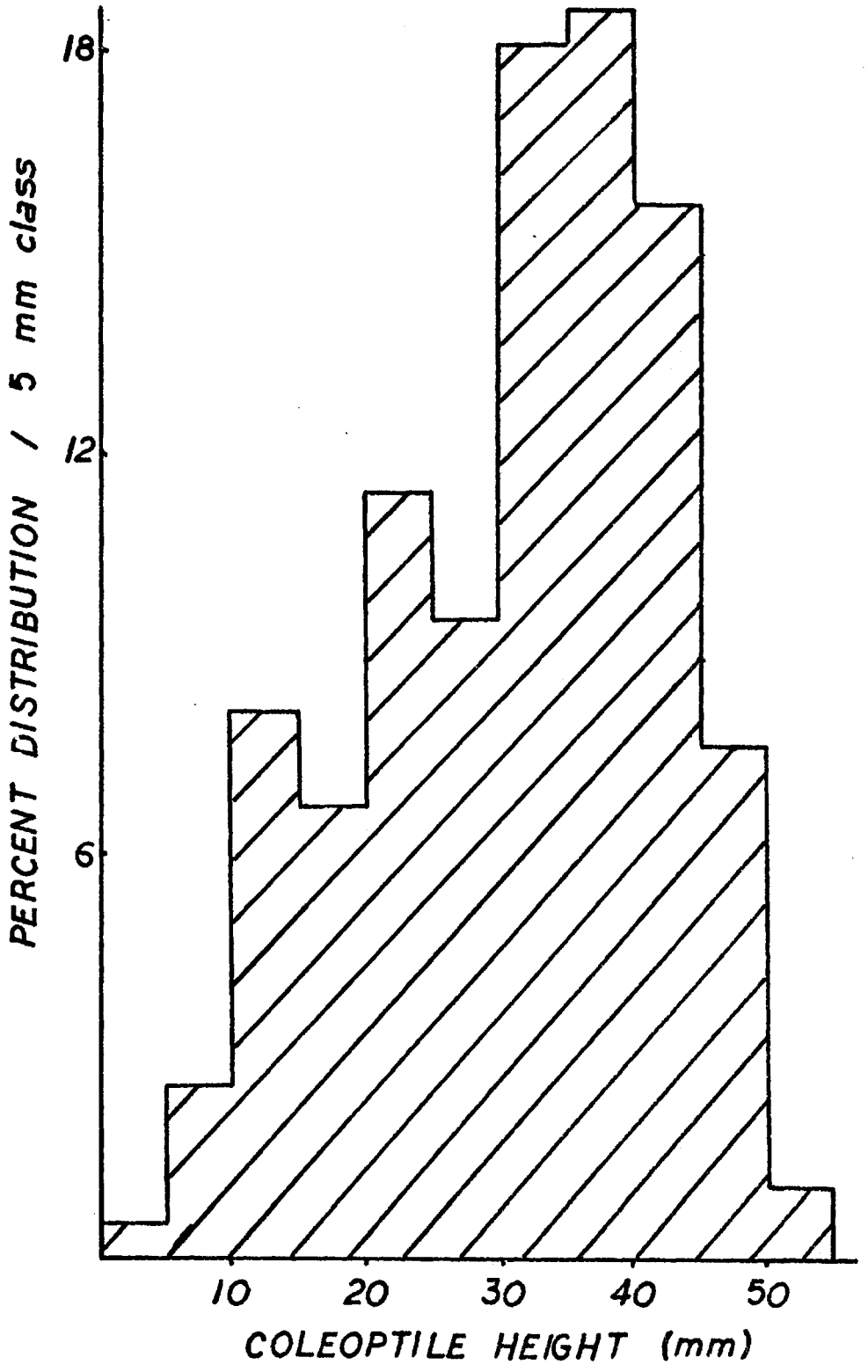
Though coleoptile growth is variable, the same overall pattern of growth is always found in replicate trials. Individual coleoptiles follow one of six patterns of growth. It has also been shown that coleoptiles of similar heights may have different growth rates and may show active growth at different times.

The conclusion from these experiments is important. Physiologically uniform coleoptiles cannot be selected solely on the basis of height at any one time in the period of germination. For experimental assays height must be rel-

FIGURE 8

Height Distribution at 96 Hr of Coleoptiles
Grown at 19 C

Seedlings were germinated in the dark at 19 C and coleoptile heights were measured at 96 hr. The histogram is based on 210 coleoptiles.


PERCENT DISTRIBUTION / 5 mm class
18
12
6
10
20
30
40
50
COLEOPTILE HEIGHT (mm)

ated to the time and rate of growth. These results also suggest that at least part of the difficulty there has been explaining how coleoptile growth is regulated lies in the fact that many workers have unwittingly used physiologically variable coleoptile samples.

5. Changes in Coleoptile Height, Fresh Weight and Dry Weight

Height increases of coleoptiles could be due solely to water uptake, though they may also involve increases in dry weight. In order to determine whether the rate of increase of height was equal to that of fresh or dry weight these weights of 20 uniform coleoptiles were measured during germination. Early in germination, i.e. at 15, 22 and 33 hr, when the various classes of coleoptiles cannot be identified with certainty, the tallest coleoptiles from seedlings with three or more roots were used while at 42, 68 and 115 hr of germination class I seedlings were selected (Table I, Fig. 9).

These selected coleoptiles elongate rather slowly for the first 33 hr of germination. From 15-33 hr the height increase is only 3.6 mm and the growth rate is 200 μ/hr. A change from slow to rapid extension occurs at about 33 hr. In the nine hr period from 33-42 hr the growth rate

TABLE I

Changes in Height, Fresh Weight and Dry Weight per Coleoptile During Germination

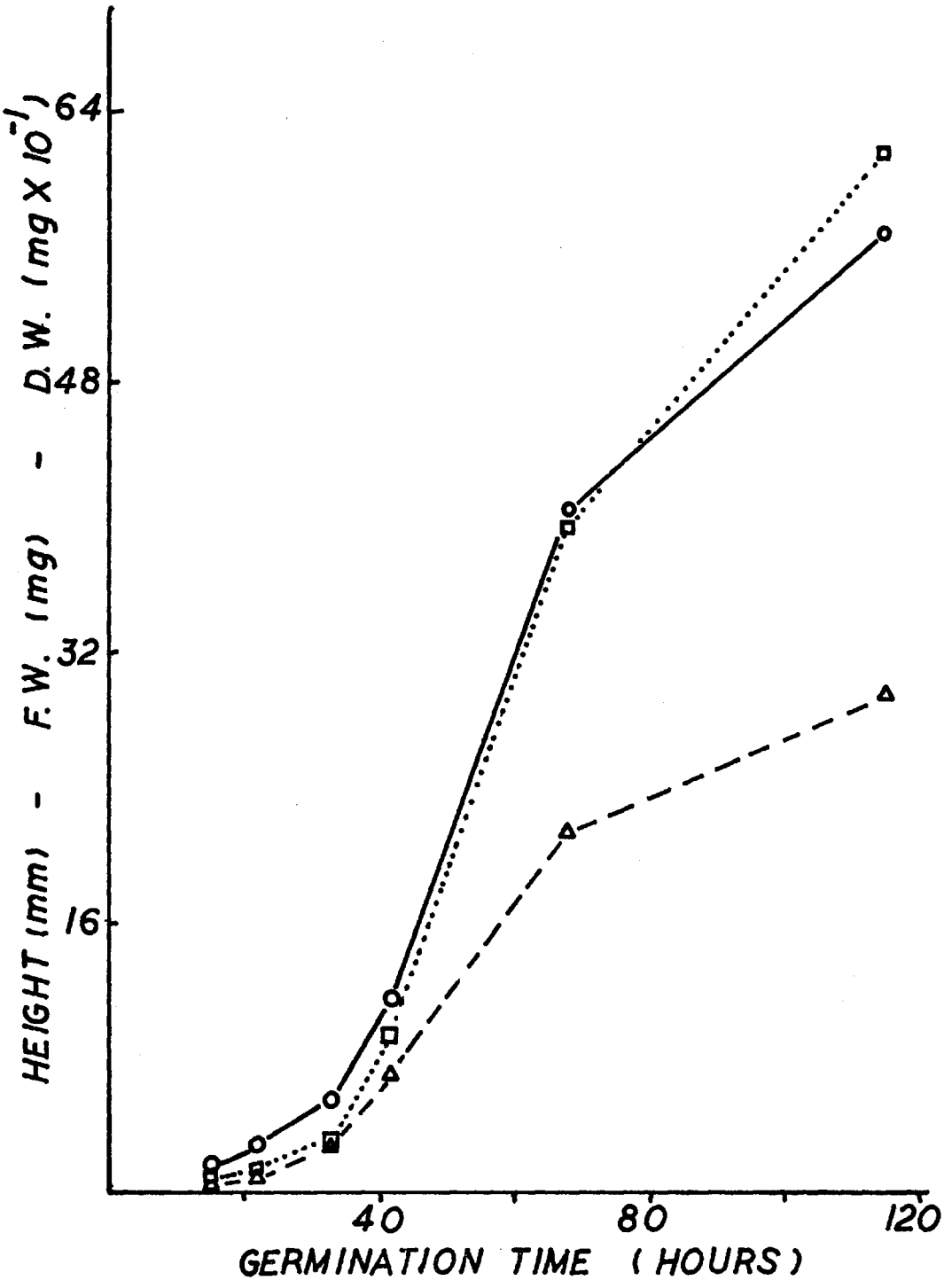
Germination (hr)	Height (mm)	Fresh Wt (g)	Dry Wt (g)
15	1.9 ± 0.29	0.63	0.04
22	3.0 ± 0.34	1.27	0.10
33	5.5 ± 0.63	3.3	0.31
42	11.7 ± 1.42	9.5	0.69
68	40.7 ± 1.98	39.8	2.17
115	57.0 ± 3.68	61.6	2.96

Twenty coleoptiles of uniform height were used for the determinations at each of the germination times. At 15, 22 and 33 hr the tallest coleoptiles with roots were used and at later times class I coleoptiles were selected. S. D. are reported for the mean heights. Fresh and dry weights were measured on the composite sample.

FIGURE 9

Coleoptile Height, Fresh Weight and Dry Weight

Changes in coleoptile height, o——o ; fresh weight, □·····□ ; and dry weight, △— —△ during germination. Until 44 hr the selected coleoptiles were the tallest from seedlings with three or more roots; at later times they were class I. Data are from the same 20 coleoptiles per germination time as in Table I.


HEIGHT (mm) - F.W. (mg) - D.W. (mg X 10⁻¹)
64
48
32
16
40
80
120
GERMINATION TIME (HOURS)

more than triples to 690 μ/hr.

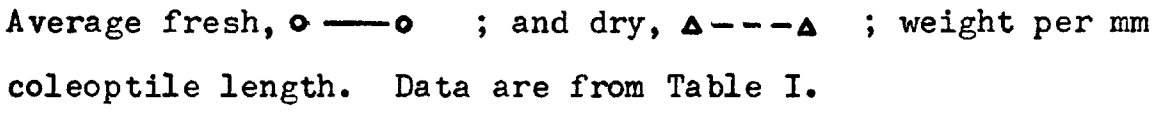
Increase in coleoptile fresh weight is relatively slow up to 33 hr, when it begins to increase rapidly (Fig. 9,10). The increase in dry weight is different; before 33 hr it is more rapid than the fresh weight increase and after 33 hr much slower. There is a 775 % increase in dry weight, compared to 524 % for fresh weight from 15-33 hr. From 33-115 hr the dry weight increases only 955 % compared to 1,869 % for the fresh weight. The dramatic change at 33 hr probably reflects a sudden shift in physiological state. It appears that coleoptile cells begin to take up much more water and accumulate relatively less dry matter after 33 hr i.e. beginning with the initiation of rapid growth. All coleoptiles however, do not begin rapid growth at exactly the same time and these changes will therefore occur at different times in coleoptiles from different classes.

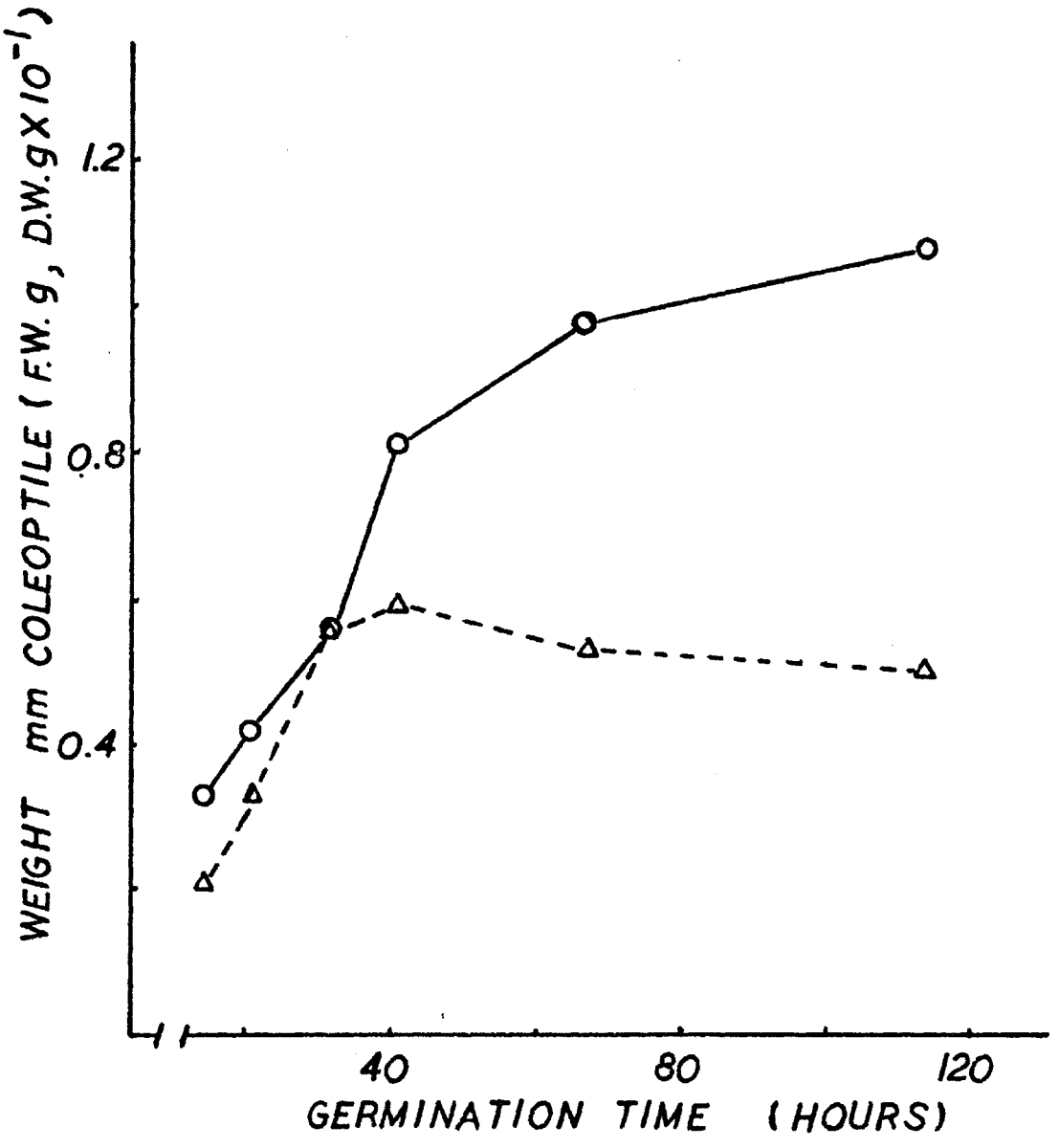
6. Coleoptile Segment Response to IAA

Since coleoptiles do not grow uniformly during germination the inherent potential for further growth appears to differ in different coleoptiles at almost any time in the main period of growth i.e. 24-90 hr (Fig. 7). This variability in potential for further growth may be reflected in variability in the response of coleoptiles to auxin (Went and

FIGURE 10

Fresh and Dry Weight per mm of Coleoptile




WEIGHT mm COLEOPTILE (F.W. g, D.W. g X 10⁻¹)
1.2
0.8
0.4
40
80
120
GERMINATION TIME (HOURS)

Thimann, 1937; Rayle et al, 1970). The object of the next experiments was therefore to determine if there was any correlation between the growth rates of coleoptiles and their response to exogenous IAA.

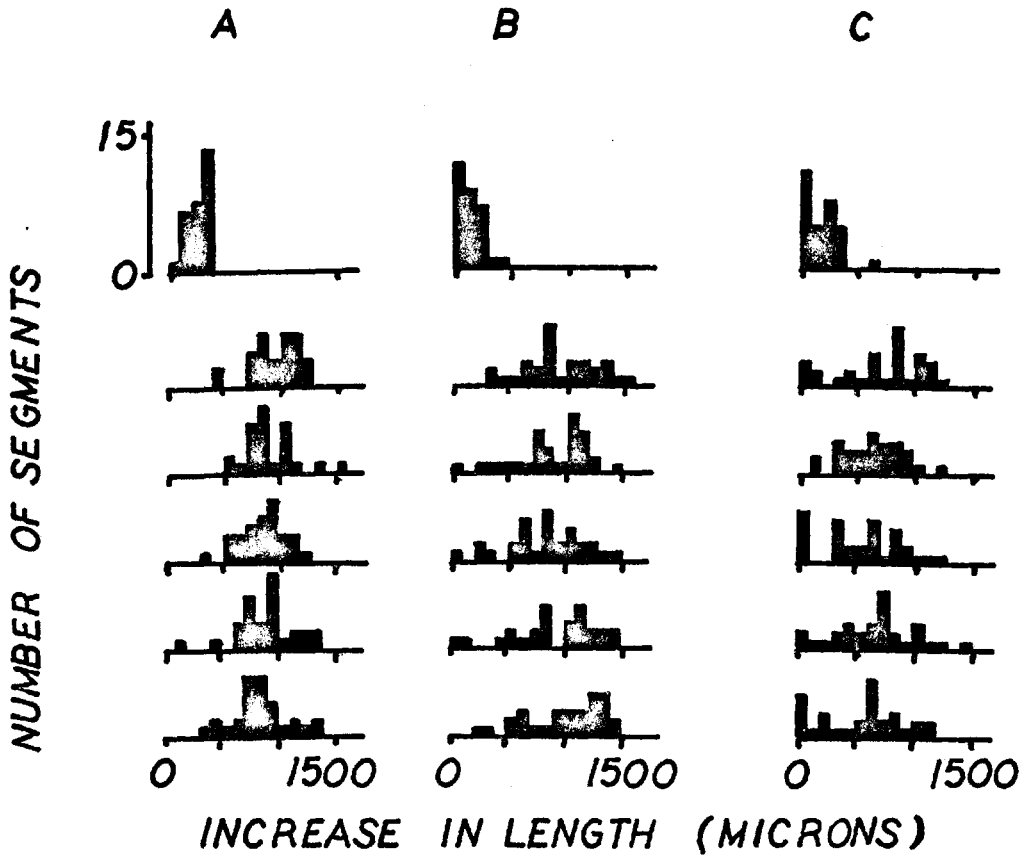
Three types of coleoptiles were selected: a) 15-20 mm at 48 hr, class I seedlings that had just begun their maximum growth rate, b) 21-26 mm at 70 hr, class II seedlings just before they separate into class IIa and IIb, and c) 15-50 mm at 75 hr, a random sample. One segment five mm long was cut from each coleoptile and floated on distilled water or 10^{-5} M IAA for three hr. Response to IAA was measured in terms of increase in segment length.

Segments from coleoptiles 21-26 mm long at 70 hr were stimulated to elongate when floated on IAA for three hr, but there was considerable variation in their response; the increase ranged from 10-1,510 μ per five mm segment (Fig. 11 A,B) and the mean increase ranged from 832 ± 323 to 1,028 ± 339 μ. The cause of this variation probably lies in the fact that all coleoptiles were not growing at the same rate. This size class of coleoptiles at 72 hr would include coleoptiles with class IIa type and others with class IIb type growth (Fig. 6) i.e. some would almost have completed their growth and some would still be growing actively. Thus, the variation in the response of coleoptile segments to IAA parallels the variation in growth rate.

FIGURE 11

Effect of IAA on Coleoptile Segment Elongation

Frequency histograms of the increases in lengths of coleoptile segments after three hr in distilled water or 10^{-5} M IAA are shown. Each segment was five mm at the time it was cut from a coleoptile. The segments were taken from selected coleoptiles: A and B, 21-26 mm long at 70 hr; C, 15-50 mm long at 75 hr. Each replicate contained 30 segments. Data for control segments, floated on distilled water, are given in the upper histogram in each vertical row.


A
B
C
15
0
NUMBER OF SEGMENTS
0
1500
0
1500
0
1500
INCREASE IN LENGTH (MICRONS)

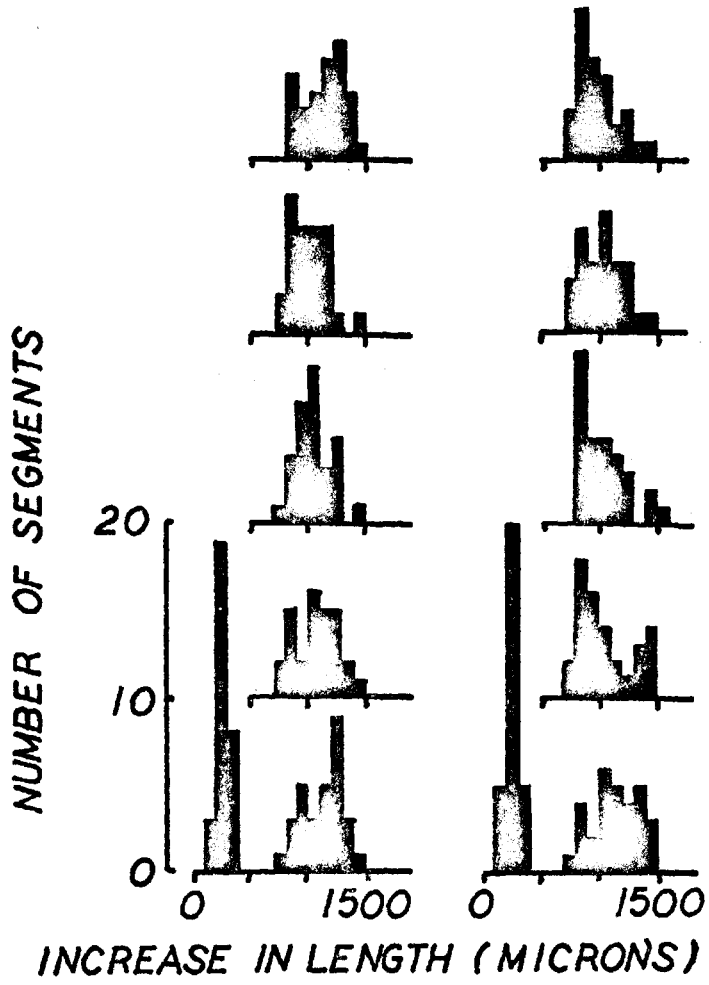
Similar variability in the extent of elongation induced in five mm segments by IAA is seen (Fig. 11C) when coleoptiles are selected at random; their heights range from 15-50 mm at 75 hr. Some segments respond to IAA and elongate while others show almost no response. Thus uniformity of height tends to give less variability in the response of coleoptile segments to IAA (cf. Fig. 11 A,B,C), but is not a truly dependable criterion for selecting coleoptiles for such a test.

At 48 hr it is known (Fig. 6,7) that coleoptiles 15-20 mm high are class I type and have a growth rate of about 870 μ/hr. The response, to IAA, of segments from these coleoptiles chosen because 1) they were known to be growing actively and 2) they were of a particular height, shows considerable uniformity. Each test sample consisted of 30 segments. In ten tests with five replicates from one set of seedlings and five from another, overlap between the lengths of control and treated segments was never observed (Fig. 12). With control segments the mean increases were 283 ± 60 μ and 265 ± 62 μ compared with mean increases in treated segments of from 990 ± 191 μ to 1,134 ± 177 μ. It is interesting that in this class of coleoptiles all segments showed some increase in length, even in distilled water, whereas in other samples (Fig. 11) some coleoptile segments showed no increase when floated on IAA.

FIGURE 12

Effect of IAA on Class I Coleoptile
Segment Elongation

Frequency histograms of the increases in lengths of coleoptile segments after three hr in distilled water or 10^{-5} M IAA. Each segment was five mm at the time of excision from a coleoptile. The coleoptile heights were 15-20 mm at 48 hr. Each sample contained 30 segments. Values for control segments, grown on distilled water, are shown to the left of each group of five replicate treatments in IAA.


NUMBER OF SEGMENTS
20
10
0
0
1500
0
1500
INCREASE IN LENGTH (MICRONS)

The results (Fig. 12) suggest that the response of coleoptile segments to IAA depends upon their growth rate at the time of treatment, not their length (cf. McCrae and Bonner, 1953). By selecting coleoptiles that are growing actively i.e. that are in a similar physiological state, a reproducible response to IAA can be obtained.

7. Cell Number in Coleoptiles

The variation in time and rate of maximum growth of coleoptiles of different classes could be a reflection of differences in time and frequency of cell division. A rapid method of estimating an increase in cell number, and indirectly of cell division in a coleoptile is to count the number of cells along its longitudinal axis. Cell numbers were determined throughout the growth period of the six subclasses of coleoptiles.

In coleoptiles taken at random at 12 hr the mean number of cells per column is 78 while in class I coleoptiles it is 88 (Table II). These values constitute, per column of cells, a base number with which all increases in cell number can be compared. They also serve as an indicator of the number of cells present in a column at the time the embryo became dormant. In coleoptiles, most of which became class I, cell number increases early in germination

TABLE II

Cell Number in Vertical Columns of Coleoptile Cells

Class	Age (hr)	Height (mm)	Cell No.	Cell Length (μ)
A	12	2.0	88 ± 6.0	22.7
A	24	4.6	132 ± 22.1	34.8
A	34	7.6	156 ± 20.2	48.7
I	44	16.6	223 ± 20.8	94.4
I	68	43.8	218 ± 15.6	200.6
IIa	78	22.2	137 ± 22.5	162.0
IIb	78	39.8	196 ± 10.1	203.1
IIIa	115	9.4	85 ± 9.7	110.6
IIIb	115	20.0	146 ± 15.6	137.0
IIIc	115	36.4	207 ± 19.1	180.1
Random	12	-	78 ± 6.5	-

Determinations are based on samples of ten coleoptiles. S. D. are reported for cell numbers. Cell lengths are calculated. A, refers to the tallest coleoptiles at 12, 24 and 34 hr from seedlings with roots.

and reaches its maximum by 44 hr; this increase occurs between 12 and 44 hr and results in a threefold rise in cell number. In the same period, there is an eightfold increase in coleoptile height (Table II). While cell proliferation stops around 44 hr cell elongation continues and between 44 and 68 hr the class I coleoptiles increase in height from 16.6 to 43.8 mm. As in other systems, cell division and cell elongation can occur independently of one another.

The number of cells per column and cell length in class I coleoptiles when division has stopped is similar to the cell numbers and cell length in class IIb and IIIc coleoptiles i.e. 218 cf. 196 and 207 cells (Table II). Coleoptiles of intermediate final height, i.e. class IIa and IIIb are also similar in number of cells and cell length. As with tall coleoptiles, the coleoptiles of intermediate final height grow at different times but eventually resemble each other. Thus, in height, 22.2 and 20.0 mm and in cell number per column 137 and 146, class IIa and IIIb coleoptiles are similar (Table II). This suggests that the same sequences of events occur in the two types of coleoptiles, but they occur at different times.

Class IIIa coleoptiles are an isolated class. They have only 85 cells per column even after 115 hr of growth. This is only seven cells more than in randomly selected coleoptiles at 12 hr (Table II) and it strongly suggests that,

at most, only two or three cells in each column have divided during the growth period. Mean coleoptile height is 9.4 mm. This represents an increase of eight mm, but it is a small increase relative to the increases found in all other coleoptiles. For these coleoptiles there has been little stimulus to undergo cell division.

The results (Table II) show that the extent of the increase in coleoptile height parallels the increase in cell number per column of cells. From these results it is clear that the six types of coleoptiles identified by their pattern of elongation can also be separated into six groups on the basis of cell number (Table II). It also appears that the restraint on elongation that limits final height of class IIa, IIIa and IIIb coleoptiles is paralleled by restraints on cell proliferation .

8. Germination of Irradiated Barley Seed

To test the possibility that the restraint on cell division is controlled by the same mechanism that regulates cell elongation it was decided to study the coleoptiles of irradiated seed. Coleoptiles from seeds irradiated with 250 Krads of gamma rays from a ^{60}Co source show no increase in cell number (Table III). This dose had previously been shown to inhibit cell division in germinating wheat embryos

TABLE III

Cell Number in Gamma-irradiated Coleoptiles at 115 Hr of Germination

Height (mm)	Cell No.	Cell Length (μ)
6	81.8 ± 5.6	73
19	80.0 ± 6.4	238
23	78.1 ± 5.2	294
32	80.2 ± 5.4	400

Cell numbers were determined on each of five coleoptiles 6, 19, 23 or 32 mm high selected from the coleoptiles of the irradiated seeds (see Fig. 14).

but allows abnormally large amounts of cell expansion (Haber, 1968). The number remains constant at about 80 cells per column. This value is similar to that found in 12 hr seedlings and class IIIa seedlings at 115 hr (Table II).

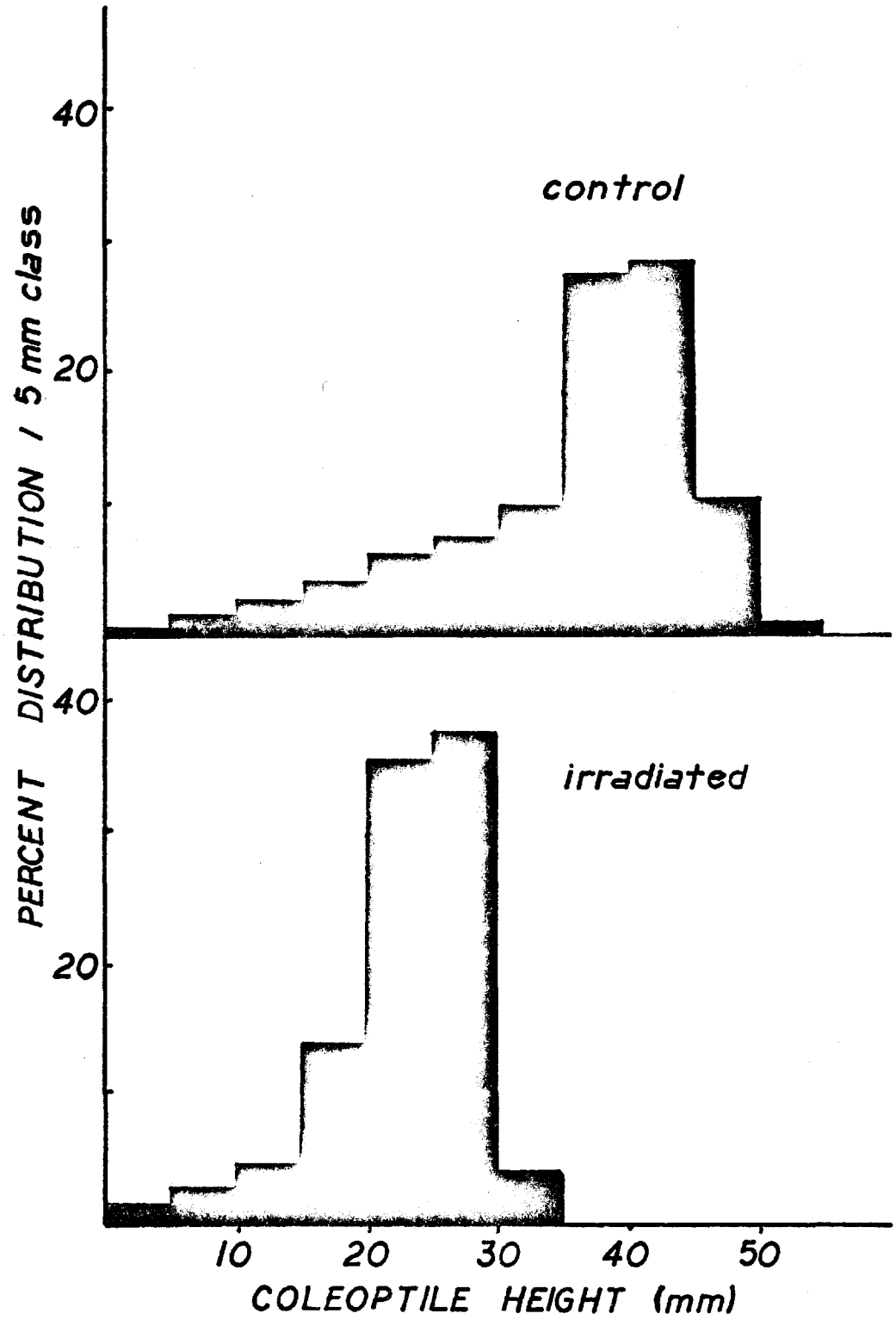
Though no increase in cell number took place, some coleoptiles grew to 32 mm by 119 hr. This compares with a maximum height of 53 mm in control coleoptiles (Fig. 13). Other coleoptiles remained short i.e. similar to class IIIa coleoptiles of unirradiated seeds, while some achieved an intermediate final height. In the irradiated coleoptiles there is, therefore, a spread of heights that is similar, though somewhat reduced in extent, to the range of values found in normal coleoptiles. Thus, although division has been suppressed the coleoptiles from irradiated seed also show variation in their final heights. That aspect of growth has not been suppressed as a consequence of irradiation.

One group of seedlings was selected 68 hr after the beginning of germination. These seedlings had coleoptiles between five and seven mm in height and were among the slow growing group of irradiated seedlings. Maximum coleoptile height in the irradiated plants was 22 mm. The seedlings with 5-7 mm coleoptiles were grown on till 119 hr. Their heights then ranged from 5-31 mm. Thus, the elongation patterns of coleoptiles of irradiated seeds are sim-

FIGURE 13

Effect of Gamma-irradiation of Dry Seed on Final Coleoptile Height Distribution

Coleoptile height at 119 hr. Distribution is shown for untreated and seeds irradiated with 250 Krads from a ^{60}Co source in 25 min. Six hundred seeds were set out initially and germination percent was similar in both cases.


40
20
control
PERCENT DISTRIBUTION / 5 mm class
40
20
irradiated
10
20
30
40
50
COLEOPTILE HEIGHT (mm)

ilar in type, though the range of heights is reduced, to those of normal coleoptiles. In some coleoptiles, growth is initiated early in germination; these become tall coleoptiles. In others, growth is initiated later and depending upon the rate and duration of growth, these coleoptiles become tall, intermediate or short in height. Irradiation of seed has the effect of reducing the final height of coleoptiles, but it does not suppress the the variation in the time when coleoptiles begin rapid growth.

One consequence of the suppression of cell division in coleoptiles of irradiated seeds is that growth involves a fixed number of cells. Coleoptiles of different height indicate different degrees of elongation of a specific number of cells. Differences in coleoptile heights in irradiated plants are the result of different amounts of cell elongation. While the coleoptiles from irradiated seed have a cell number identical with that of normal coleoptiles at 12 hr of germination (Table II, III), cell lengths are much greater in irradiated coleoptiles; this is similar to the results reported for coleoptiles of gamma plantlets (Haber, 1968). In coleoptiles of intermediate height i.e. 19-23 mm, calculated mean cell length is 238 and 294 μ, which is higher than the calculated mean cell lengths for cells of class I coleoptiles, 200 μ, or class IIIb coleoptiles, 203 μ, (Table II). In tall coleoptiles of irradiated

seed calculated cell length, 400 μ, is double the mean value for cells of unirradiated coleoptiles (Table III). This result means that 1) even after irradiation, the coleoptile cells have a potential for elongation that is equal to or perhaps is greater than the growth potential of normal coleoptile cells, 2) there is a supply of endogenous growth factors in irradiated coleoptiles that induces an amount of cell elongation that exceeds that found in normal coleoptile cells.

9. Protein and Free Amino Acid Levels of Coleoptiles

It is now clear that the growth patterns shown by populations of coleoptiles indicate variation in the rates of a number of growth processes. Of greatest significance are the patterns of a) onset and extent of cell proliferation and b) increase in dry weight. Particularly in the case of cell proliferation, protein synthesis must be involved. Together with cell proliferation increases in amounts of protein are more informative parameters of growth than increases in height, fresh weight or dry weight. The latter do not distinguish between water uptake or cell wall synthesis and an increase in the functional capacity of cells. The pattern of cell proliferation in different coleoptiles suggests that the underlying mechanisms of regulation are

expressed to different degrees in different classes of coleoptiles. Protein synthesis, it is suggested, should show a similar pattern of regulation; that is, there should be a close correlation between protein content and growth pattern. In order to test this a comparison was made of total and protein amino acid content of coleoptiles of various classes throughout their growth period.

Compositions and levels of protein and free amino acids were found to differ in 1) coleoptiles growing actively vs those growing slowly and 2) coleoptiles that initiate active growth at different times (Table IV).

A comparison was made between the tallest coleoptiles present between 12 to 34 hr and class I coleoptiles from 44 to 85 hr; protein amounts increase 11.5 X, height increases 34.2 X and fresh weight 80 X from 12 to 85 hr (Table IV). Not only are there differences in the absolute increments, but also the rates of individual growth parameters are different at various times during growth. For example, after 34 hr there is comparatively less protein synthesis and more water uptake than before 34 hr. This observation shows that the regulation of protein synthesis changes during growth of class I Coleoptiles. The analysis was therefore extended to other classes.

Amino acids in the alcohol soluble fraction appear to remain constant in quantity between 12 and 24 hr of germ-

TABLE IV

Height, Fresh Weight and Amino Acid Content per Coleoptile

Age (hr)	Class	Height (mm)	F,W, (mg)	Amino Acids (mμmoles/coleoptile) Alcohol Soluble	Alcohol Insoluble
12	A	1.8	0.7	42	235
18	A	2.6	0.8	37	313
24	A	3.4	1.8	39	459
34	A	7.3	4.5	64	854
44	I	15.2	10.2	277	1282
68	I	42.1	40.0	781	2154
85	I	61.6	56.0	1232	2707
85	IIIa	8.1	3.6	76	464
85	IIIb	17.1	9.6	170	687
85	IIIc	28.3	20.4	427	1109

Each determination was done on ten coleoptiles (see Methods and Materials). A, refers to the tallest coleoptiles at the indicated times.

ination (Table IV). Since the alcohol insoluble fraction doubles in this period, this probably reflects incorporation of amino acids into protein at the same rate as free amino acids are synthesized in or transported to the coleoptile. The total content of free amino acids in the coleoptile begins to increase after 24 hr of germination and by 85 hr is 31.6 times greater than at 24 hr. This represents about a threefold greater increase in free than in the alcohol insoluble amino acids. Even though the coleoptile is near the end of its growth by 85 hr of germination it contains 1,232 mμmoles of free amino acids while the total amount incorporated into the alcohol insoluble fraction by 85 hr is 2,707 mμmoles. Since after 85 hr, there is little further protein synthesis, the free amino acids could accumulate as a storage reserve for subsequent growth of the plumule.

All coleoptiles do not have similar types of growth. One class of coleoptiles, III, grow very slowly for the first 66-70 hr of germination and subsequently subdivide into three subgroups, IIIa, IIIb, and IIIc. Besides differences in growth rates and final heights in these three subgroups there are differences in amino acid contents (Table IV).

Even at 85 hr, class IIIa seedlings have only 464 mμmoles per coleoptile in the alcohol insoluble fraction.

This is comparable to the level in class I at 24 hr. Of class III coleoptiles, the tallest, IIIc, have the highest level of alcohol insoluble amino acids (Table IV). Class IIIc are 28.3 mm high and have 1,109 mμmoles of alcohol insoluble amino acids per coleoptile at 85 hr. This is less than class I at 44 hr even though those coleoptiles are 13.3 mm shorter at 44 hr than IIIc coleoptiles at 85 hr. Though both I and IIIc become tall, their amino acid levels at a particular height are not alike. Not only are there differences in total amino acid contents, but also in percentages of individual alcohol insoluble amino acids in the two types of coleoptiles. For example, the highest percentage of glycine in the protein fraction in class I is 12.9%. Class IIIc have 20.2 % of their alcohol insoluble fraction as glycine at 85 hr (Table V).

The alcohol insoluble amino acid composition of class I coleoptiles remains constant over their growth period. Changes in amount are found with lysine, arginine and aspartate-asparagine (Table V). As coleoptiles increase in height these amino acids decrease in amount. At 85 hr lysine and arginine are also less abundant in IIIc, the tallest, than in IIIb or IIIa coleoptiles. In class IIIa 12.0 % of the alcohol insoluble fraction is arginine compared to 4.3 % for IIIb, 1.1 % for IIIc or 3.8 % for I. Leucine levels of IIIa coleoptiles unlike arginine, are

TABLE V

Alcohol Insoluble Amino Acid Content per Coleoptile

Germination Time (hr)	24	68	85	85	85
Class	A	I	IIIa	IIIb	IIIc
	Percent of Alcohol Insoluble Fraction				
lysine	9.0	4.7	8.7	6.7	3.9
arginine	5.0	3.8	12.0	4.3	1.1
aspartate-asparagine	7.8	3.4	6.3	9.8	9.9
glycine	11.9	12.9	10.0	10.3	20.2
leucine	9.1	11.5	2.3	9.5	8.5

Data are from the same coleoptiles as in Table IV.

low at 85 hr, 2.3 % compared to values between 8.5 and 11.5 % for the other classes.

Thus coleoptiles that have initiated a growth rate greater than 400 μ/hr (class I, IIIb, IIIc) have a relatively low percentage of protein arginine, less than 5 %, and high levels of leucine, greater than 8.5 %. In contrast, those that do not grow actively at all, IIIa, have a high arginine and low leucine content. It is clear that coleoptiles with different rates and times of growth do not have similar protein compositions.

The most striking change in the alcohol soluble amino acid fraction is the increase in serine in class I (Fig. 14). Serine accounts for 25.7 % of the total free amino acids at 24 hr. This rises to 59.9 % at 68 hr and 61.1 % at 85 hr. The significance of the high free serine levels is considered in the Discussion (Section 7). The only other free amino acids that change substantially are glutamate-glutamine and alanine (Fig. 14). The percentages of these rise from 12 to 18 hr and then steadily decline as the serine concentration rises. Glutamate-glutamine decreases from 24.5 % of the total fraction at 18 hr to 4.2 % at 85 hr while alanine changes from 16.1 to 2.1 % during the same time.

In class I serine increases from 10.1 mμmoles per coleoptile at 24 hr to 754 mμmoles at 85 hr (Table VI).

FIGURE 14

Changes in Some Free Amino Acids of Coleoptiles During Germination

Percentage change in free serine; o——o , glutamate-glutamine; Δ——Δ , and alanine; □——□ , during germination in class I coleoptiles. Each sample was a determination on ten coleoptiles.

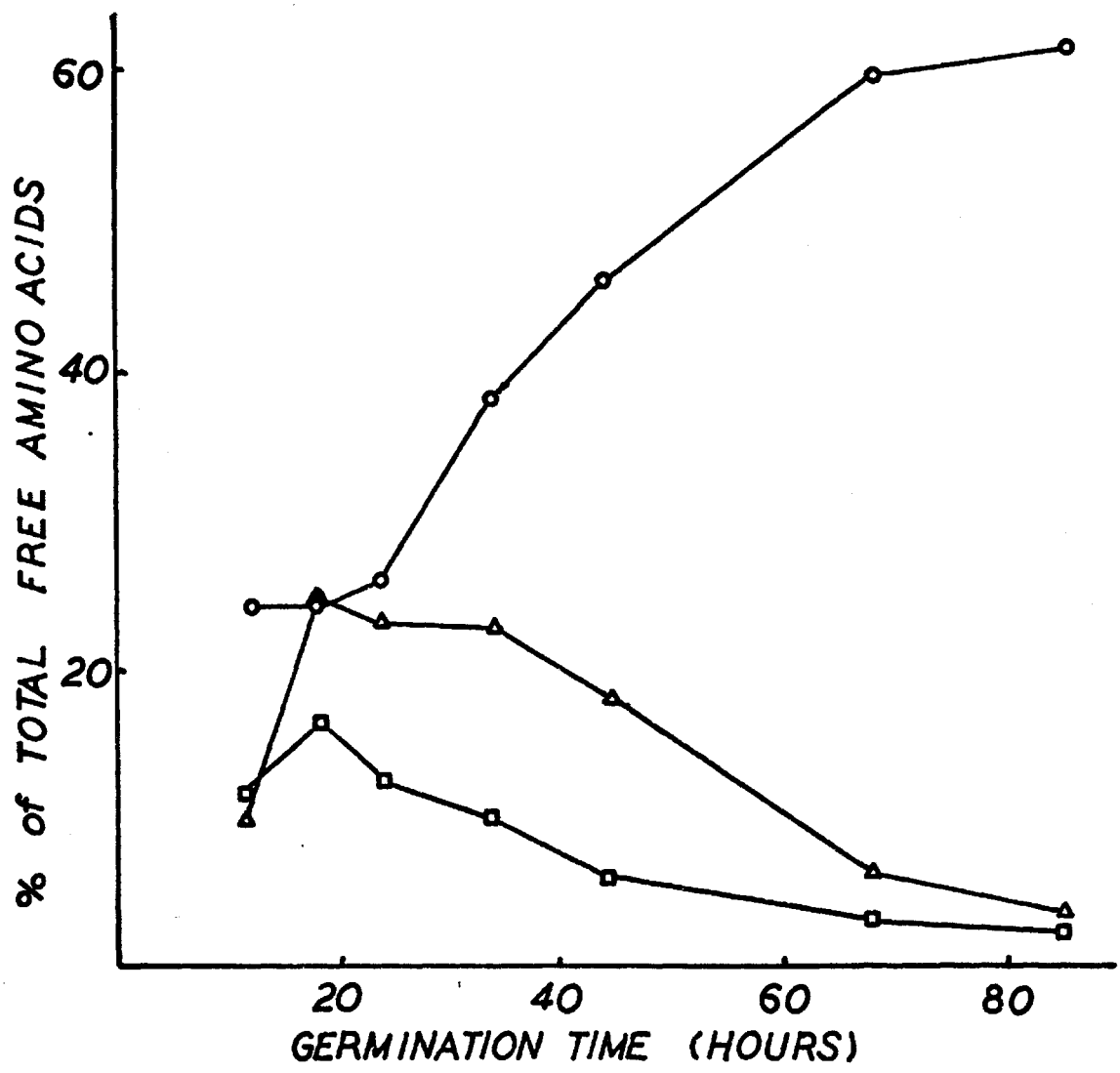

60
40
20
% of TOTAL FREE AMINO ACIDS
20
40
60
80
GERMINATION TIME (HOURS)

TABLE VI

Alcohol Soluble Serine Levels per Coleoptile

Coleoptile Age (hr)	Class	Serine Concentration (mμmoles/coleoptile)
12	A	10.1
18	A	9.0
24	A	10.1
34	A	24.2
44	I	128.8
68	I	467.7
85	I	754.1
85	IIIa	13.2
85	IIIb	93.2
85	IIIc	249.1

Data are from the same coleoptiles as in Table IV.

Both IIIb and IIIc coleoptiles also have relatively high serine levels once they have initiated active growth. Over 50 % of their free amino acids is made up of serine although class IIIb has only 93.2 and IIIc 249 mμmoles per coleoptile at 85 hr. In contrast IIIa have a low serine level i.e. 13.2 mμmoles per coleoptile. In these coleoptiles serine makes up only 17.4 % of the free amino acids. Serine thus constitutes over 50 % of the free amino acids in coleoptiles that have initiated a rapid, I and IIIc, or a medium, IIIb, but not a slow rate of growth, IIIa.

Variations between replicates of the alcohol insoluble amino acid fractions at 44 hr of germination in class I coleoptiles is small (Table VII). The basic fraction is the most variable, the other levels are fairly constant from one replicate to another. The data, which are single determinations each made of ten coleoptiles, therefore appear to be a fairly reliable estimate of coleoptile protein levels. Somewhat greater variation was seen in the amino acid levels of the alcohol soluble fraction (Table VIII). At about 44 hr class I coleoptiles are nearing their maximum rate of growth and therefore even slight differences in the stage of development between samples would be expected to affect free amino acid values in replicate determinations.

The results, (Sections 1-9), show that there is a correlation between coleoptile protein level, cell number and height. Class IIb and IIIc coleoptiles grow actively

TABLE VII

Replicates of 44 Hr Class I Coleoptile Alcohol Insoluble Amino Acid Levels (mμmoles/coleoptile)

Replicate	1	2	3	mean	S.D.
threonine	66.7	55.4	61.9	61.3	14.1
serine	85.6	86.3	99.5	90.5	7.8
proline	73.8	71.0	57.1	67.3	8.9
glycine	160.0	114.1	139.4	137.8	23.0
alanine	147.8	122.1	145.3	138.4	14.2
valine	84.5	87.1	93.1	88.2	4.4
methionine	2.3	0.6	0.5	1.1	1.0
iso-leucine	57.9	64.5	64.5	62.3	3.8
leucine	123.6	77.5	119.5	106.9	25.5
tyrosine	36.8	29.2	30.9	32.3	4.0
phenylalanine	49.8	35.3	42.0	42.4	7.3
aspartate-asparagine	87.6	115.4	97.3	100.1	14.1
glutamate-glutamine	137.0	120.8	140.2	132.7	10.4
lysine	96.9	35.3	39.3	57.1	34.5
histidine	19.2	9.0	4.9	11.0	7.4
arginine	52.4	33.4	62.0	49.3	14.6
Total	1282	1057	1197	1179	113.5

TABLE VIII

Replicates of 44 Hr Class I Coleoptile Alcohol Soluble Amino Acid Levels (mμmoles/coleoptile)

Replicate	1	2	3	S.D.
threonine	12.7	2.7	23.5	13.0
serine	128.8	91.6	110.9	18.6
proline	1.8	2.6	1.0	0.8
glycine	3.3	6.3	6.6	1.8
alanine	16.1	25.0	25.9	5.4
valine	19.1	25.2	34.0	7.5
methionine	-	0.1	0.5	0.3
iso-leucine	9.1	9.6	18.5	5.3
leucine	7.7	9.1	16.6	4.8
tyrosine	-	0.3	1.3	0.7
phenylalanine	2.4	0.3	1.3	1.1
aspartate-asparagine	11.7	75.5	124.9	57.0
glutamate-glutamine	49.6	90.7	116.4	33.7
lysine	7.5	1.8	1.2	3.3
histidine	8.0	4.7	2.4	2.8
arginine	-	0.3	-	0.2
Total	277.3	345.9	484.9	105.8

and show an increase in protein content later than class I coleoptiles. Similarly, class IIIa coleoptiles show little growth and only a slight increase in protein content. This restriction on growth in the seedling is generally confined to the coleoptile since some class III coleoptiles have long roots. Thus, it appears that there is some factor operating largely, or only, in coleoptiles that regulates the amount of growth they undergo. The data, particularly from determinations, for example, of amounts of RNA, would confirm that different classes of coleoptiles are not biochemically identical. However, such differences as those seen with protein content do not provide an explanation of differential gene expression in different coleoptiles.

The mechanism underlying differential growth in populations of coleoptiles must lie, ultimately, at the level of gene expression. Since gross changes in the amounts of various constituents do not control gene activity, but must be the result of it, it did not appear that further analysis of coleoptile constituents would lead, in a reasonable time, to an understanding of the regulation of coleoptile growth. Subsequent experiments therefore were directed to an analysis of the response of coleoptiles to various growth factors. It seemed possible that variation in endogenous levels of hormones might be the cause of variation in coleoptile growth. The response of coleoptiles to

exogenous kinetin, gibberellin, IAA or inositol at various times in germination was tested.

10. Gibberellin and Kinetin Treatments

Class I coleoptiles begin growth early in germination, some do so at later times, while IIIa never achieve an active rate of growth. Growth may be delayed or retarded in these latter coleoptiles because they lack some necessary growth factor. Exogenous application of a growth factor should then stimulate these coleoptiles to grow actively. Two growth factors that have been shown to stimulate wheat coleoptile elongation are kinetin and gibberellin (Wright, 1961). Kinetin has also been regarded to initiate cell division, especially in callus culture (Miller et al., 1956); gibberellin stimulates cell elongation (Lockhart, 1965). These growth factors were therefore supplied to a population of germinating barley seeds in an attempt to stimulate active growth in all coleoptiles simultaneously.

Barley was germinated and grown on 12 ml of distilled water, 10^{-5} M gibberellin or 10^{-5} M kinetin. The percentage germination was similar in all cases: 70 % on water, 66.3 % on kinetin and 70.3 % on gibberellin. Coleoptile heights of seedlings grown on water for 66 hr did not exceed 40 mm (Table IX), but with gibberellin or kinetin, some coleoptiles grew to 46-50 mm. The frequency of coleoptiles

TABLE IX

Effect of Continuous Gibberellin or Kinetin Treatment on Coleoptile Height

Coleoptile Height at 66 Hr (mm)	Percent Distribution		
	Control	$GA_3(10^{-5}M)$	Kinetin (10^{-5})
2-5	4.8	1.4	0.8
6-10	6.4	3.6	2.0
11-15	8.1	5.2	3.3
16-20	19.0	12.8	8.0
21-25	16.9	10.9	9.5
26-30	23.3	18.0	21.6
31-35	18.1	27.3	26.1
36-40	3.3	15.6	23.1
41-45		4.7	4.5
46-50		0.5	1.0

Six hundred seedlings were used for each treatment. Coleoptile heights were measured at 66 hr of germination.

30-50 mm high was greater after treatment with gibberellin, 48.8 %, or kinetin, 54.7 % than after growth on distilled water, 21.4 % (Table IX). A stimulation of growth also occurs but only in some short coleoptiles. The 2-20 mm height class constitutes 38.3 % of coleoptiles of untreated seedlings, but it is only 23.0 % after gibberellin and 14.1 % after kinetin treatment.

Continuous treatment with 10^{-5} M gibberellin or kinetin clearly stimulates coleoptile elongation; the frequency of short coleoptiles shows a corresponding reduction. Coleoptiles of intermediate height have probably contributed to the class of tall coleoptiles and in turn, after treatment with one of the growth factors, include short coleoptiles that were stimulated to elongate. However, not all short coleoptiles have been stimulated to become tall by 66 hr. Though both growth factors stimulate tall and some short coleoptiles to elongate, their effect is not uniform i.e. the coleoptiles vary in their ability to respond to gibberellin or kinetin. The stimulatory effect of these compounds appears to be mainly on coleoptiles with a relatively rapid rate of growth in the first 30-60 hr of germination i.e. those that grow at least 400 μ/hr. Thus, coleoptiles that are growing actively are stimulated to elongate by gibberellin or kinetin, but neither compound induces coleoptiles that are growing very slowly to begin rapid elon-

gation. Even continuous treatment for 66 hr does not convert all short slowly growing coleoptiles into rapidly elongating ones.

With kinetin treatment there are more tall coleoptiles i.e. greater than 30 mm high, and fewer short coleoptiles, less than 20 mm high, than after gibberellin (Table IX). This suggests that, in the period up to 66 hr, seedlings are more sensitive to kinetin than to gibberellin. This possibility was tested by using selected seedlings and treating for short periods.

11. Pulse Treatment of Seedlings With Kinetin

To obtain a sample of coleoptiles that were more uniform in their growth pattern seedlings that had three or more roots were selected at 24 hr. Only 2.7 % of the coleoptiles were less than 20 mm high compared to 38.3 % of the population of the unselected seedlings. A greater percentage of coleoptiles were in the 25-45 mm range in the selected group, 87.3 % (Table X), than in the unselected seedlings, 44.7 % (Table IX). Therefore, the selected group of seedlings constitute a much more uniform population of coleoptiles; most of them show an early rapid growth and develop into tall coleoptiles.

The stimulation of growth of selected seedlings by kinetin is similar in one aspect to the stimulation seen

TABLE X

The Effect of Kinetin on Selected Coleoptiles

Coleoptile Height (mm)	Percent Distribution at 68 Hr				
	Time of Kinetin Treatment (hr)				
	Control	24-34	34-44	44-54	24-68
1-20	2.7	8.7	0.7	2.0	4.0
21-25	10.0	8.7	2.0	3.3	4.0
26-30	37.3	22.7	16.0	26.0	10.0
31-35	32.7	31.3	34.7	44.0	26.7
36-40	15.3	24.0	36.0	23.3	40.7
41-45	2.0	4.0	8.0	1.3	14.7
46-50		0.7	2.7		

One hundred and fifty seedlings having three or more roots at 24 hr were selected for each treatment. Seedlings were grown in 12 ml of kinetin ($3X10^{-6}M$) for each of the indicated times after which they were returned to water. Coleoptile heights were measured at 68 hr.

with the unselected seedlings. All coleoptiles are not stimulated to elongate. At least 2.7 % remain less than 25 mm high by 68 hr of germination (Table X). Of the three different ten hr treatments i.e. 24-34 hr, 34-44 hr, and 44-54 hr, kinetin was most effective in promoting coleoptiles to elongate in the 34-44 hr period. With this treatment only 2.7 % of the coleoptiles are less than 25 mm high and 81.4 % are from 31-50 mm (Table X). The same size classes in control coleoptiles made up 12.7 % and 50 % of the coleoptiles. The other short-term kinetin treatments, 24-34 hr or 44-54 hr are less effective than the 34-44 hr treatment in reducing the frequency of short coleoptiles (Table X).

Treatment with kinetin from 24-68 hr affects both short and tall coleoptiles, though with different results. There are more short coleoptiles, less than 25 mm, with this treatment, 8.0 %, than with the 34-44 hr, 2.7 %, or 44-54 hr, 5.3 %, treatments. There are however, as many long coleoptiles, 31-50 mm with a 24-68 hr treatment, 82.1 %, as with a 34-44 hr treatment, 81.4 %. Though there are as many tall coleoptiles with the 24-68 hr as with the 34-44 hr treatment, some coleoptiles are actually retarded by the longer treatment. This slight inhibitory effect of the 24-68 hr treatment probably takes place between 24-34 hr since the 24-34 hr kinetin treatment results in the greatest frequency of short coleoptiles, 17.4 %.

Most of the selected seedlings used for these kinetin treatments initiate rapid growth at about 34 hr of germination. The greatest stimulation induced by kinetin is found in coleoptiles that began to elongate rapidly at this time i.e. 34 hr. A kinetin treatment from 24-68 hr of germination probably stimulates elongation of coleoptiles that grow actively between 34-44 hr. But kinetin also holds back the shortest coleoptiles in the 24-34 hr period. Thus, 1) coleoptiles must initiate rapid growth before they are competent to respond to kinetin and 2) kinetin does not induce slow growing coleoptiles to begin rapid growth and, indeed may even retard them.

12. Response of Coleoptiles to Gibberellin

Seedlings, with three or more roots, were selected at 24 hr and treated with a 10^{-5} M gibberellin solution. Treatments between 24-34 or 34-44 hr have little effect on the frequency distribution of coleoptile heights (Table XI). Continuous treatment from 24-68 hr, however, reduces the frequency of coleoptiles that are less than 25 mm high. There are 4.7 % of the treated coleoptiles in this height range compared to 10.0 % of the control coleoptiles. Gibberellin also increases the frequency of tall coleoptiles, i.e. those from 36-50 mm, to 50.7 %, from 36 % in controls.

TABLE XI

The Effect of Gibberellin on the Elongation of Selected Coleoptiles

Coleoptile Height (mm)	Percent Distribution (68 Hr)					
	Control	Time of GA_3 Treatment				
		24-34	34-44	24-68	34-68	44-68
1-20	6.0	6.0	2.0	2.0	2.0	1.3
21-25	4.0	4.7	6.7	2.7	3.3	3.3
26-30	14.0	22.7	13.3	12.7	13.3	11.3
31-35	40.0	38.7	40.7	32.0	30.0	24.0
36-40	29.3	23.3	34.7	39.3	40.0	38.0
41-45	6.7	4.7	2.7	10.7	10.7	21.3
46-50				0.7	0.7	0.7

Seedling selection and treatments were as in Table X

The greatest increase in the frequency of 36-50 mm high coleoptiles occurs with a gibberellin treatment from 44-68 hr of germination. Tall coleoptiles, 36-50 mm, make up 60 % of the total sample; this compares to 36 % for the control seedlings. This treatment also has the fewest number of coleoptiles in the short class, i.e. less than 30 mm.

A comparison of the response of coleoptiles to gibberellin treatments at different times indicates that gibberellin does stimulate growth of some coleoptiles, but that it is effective only when given later than 44 hr from the beginning of germination. The greatest increase in the frequency of tall coleoptiles, 36-50 mm, occurs after treatment from 44-68 hr. It is also seen that gibberellin stimulates coleoptile growth immediately after the period in which they would have shown their greatest response to kinetin.

An exogenous gibberellin treatment of barley seedlings either continuously up to 66 hr (Table IX), or from 24-68 hr (Table XI), stimulates extension of some coleoptiles. In the light of previous evidence that gibberellin stimulates seedling growth, one interesting result of the present work is that it does not induce slowly growing coleoptiles to begin rapid extension. This aspect of the response of barley to gibberellin was examined by selecting slowly growing seedlings and treating them with 10^{-5} M and 10^{-4} M solutions of gibberellin.

After 24 hr on distilled water, seedlings were selected in which only the coleorhiza had emerged; no roots were visible. They were treated for 10 hr with gibberellin, rinsed with distilled water and then grown till 66 hr in dishes containing distilled water. This 10 hr gibberellin treatment of seeds, whose germination is slow, reduces the mean coleoptile height at 66 hr (Table XII). Gibberellin also inhibits the increase in fresh and dry weights of the developing coleoptile and plumule. These results confirm that barley seeds that are naturally slow to germinate and to begin rapid coleoptile growth are not stimulated to grow by gibberellin, but are actually retarded. A stimulatory effect of gibberellin is seen much later in seedling growth i.e. after 44 hr and mainly on coleoptiles that have already achieved a rapid growth rate in the absence of any exogenous growth factor.

Neither kinetin nor gibberellin is able to initiate active growth in slowly growing coleoptiles, though in the case of gibberellin, uptake occurred since an inhibition of growth was observed. Both compounds can, however, promote elongation once active growth of coleoptiles has been initiated; stimulation occurs at different times with the two compounds. The failure to induce active growth suggests, therefore, that these compounds do not have true hormonal properties in this test system i.e. they are incapable of

TABLE XII

The Effect of Gibberellin Supplied at 24-34 Hr on Shoot Growth of Slowly Germinating Seedlings

Treatment	Coleoptile Height (mm)	Height Range	Combined Coleoptile & Plumule F.W.(mg)	D.W.(mg)
Control	17.4 ± 3.6	1-28	23.0	1.70
GA_3 (10^{-5}M)	16.1 ± 7.4	1-26	19.6	1.44
GA_3 (10^{-4}M)	13.3 ± 5.4	1-22	16.9	1.35

One hundred seedlings having only the coleorhiza emerged at 24 hr of germination were treated from 24-34 hr with 10^{-5} or 10^{-4} gibberellin, then grown till 68 hr in distilled water at which time the above determinations were performed.

initiating growth. It appears instead that they act as modulators or "enhancers" of processes that had previously been initiated by other mechanisms.

13. Response of Coleoptiles to Inositol and IAA

Two other growth factors that have been reported to stimulate plant growth are inositol and IAA (Steward et al, 1969). The ability of these compounds to induce uniform growth in populations of coleoptiles have also been tested.

An unselected sample of seeds was germinated in dishes containing 12 ml of Inositol or IAA. The IAA treatment reduced the frequency of coleoptiles greater than 55 mm high by 10 % and increased the frequency of coleoptiles less than 50 mm by 6 % (Table XIII cf. IAA and controls). Inositol, by contrast increased the frequency of tall coleoptiles, greater than 55 mm, by 10.7 % and there was a corresponding 9.3 % decrease in the frequency of shorter coleoptiles (Table XIII).

Barley seedlings that had developed three or more roots by 24 hr were transferred to dishes containing inositol and given continuous treatment until 68 hr. Seedlings on 10^{-6} M inositol had a greater percentage of tall coleoptiles and fewer short coleoptiles than controls (Table XIV);

TABLE XIII

Effect of Inositol and IAA on Coleoptile Growth

Coleoptile Height (mm)	Percent Distribution		
	Control	IAA(10^{-5})	Inositol($3X10^{-6}$)
1-40	5.4	9.3	3.4
41-45	15.3	12.0	5.3
46-50	19.3	24.7	22.0
51-55	28.9	32.7	27.3
56-60	27.3	17.3	28.7
61-65	3.3	4.0	9.3
66-70	0.7		4.0

Six hundred unselected seedlings were used for each treatment. Coleoptile heights were measured at 82 hr.

TABLE XIV

Effect of Inositol on Coleoptile Growth

Coleoptile Height (mm)	Percent Distribution			
	Control	Inositol 10^{-2}	10^{-4}	10^{-6} M
1-20	8.7	8.7	7.3	7.3
21-25	5.3	6.7	7.3	5.3
26-30	17.3	21.3	21.3	10.0
31-35	42.7	34.7	42.7	37.3
36-40	23.3	23.3	19.3	30.0
41-45	2.7	5.1	2.0	8.7
46-50				1.3

One hundred and fifty seedlings having three or more roots at 24 hr were selected for each treatment. Coleoptile heights were measured at 68 hr.

40 % were greater than 36 mm and 22.6 % were less than 30 mm compared with 26.0 % and 31.3 % in control seedlings. With 10^{-2} and 10^{-4} M solutions coleoptile heights were rather similar to those of controls (Table XIV).

Thus IAA inhibits coleoptile elongation. This inhibition may result from an indirect effect on the coleoptile following inhibition of root growth. It does not necessarily mean that IAA taken up by roots and transported to coleoptiles is producing a direct inhibition; nevertheless, IAA available to coleoptiles through the root is failing to produce the stimulation reported for excised coleoptiles by Wright (1961). Unlike IAA, the others, inositol, kinetin, and gibberellin can stimulate coleoptile elongation when supplied to roots.

In terms of response to these growth factors it is interesting that a 24-68 hr continuous treatment with 10^{-6} M inositol is almost as effective as 3×10^{-6} M kinetin (Table X and XIV). Inositol, moreover, like kinetin and gibberellin, does not induce active growth in coleoptiles that are growing slowly. Initiation of active growth may require a combination of exogenous growth factors. In a pilot experiment, however, a combination of kinetin and gibberellin produced no greater effect than either compound alone; these experiments were not pursued. None of the four known growth factors that have been tested here has stimulated

growth of slowly growing coleoptiles. It is possible that either i) some other growth factor must be supplied to trigger active growth or ii) growth is regulated by a common intermediate or end product of metabolism. One compound that has been shown to affect plant growth is CO_2. Since CO_2 produced by respiration accumulates in the coleoptile chamber, it may affect growth. This possibility was tested in the experiments described in the following section.

14. CO_2 Effects on the Time of Plumule Emergence Through the Coleoptile

CO_2 can either stimulate or inhibit plant growth. In particular, it has been reported to stimulate coleoptile segment elongation (Rayle and Cleland, 1970) and inhibit root and shoot growth (Mer, 1957; Stolwijk and Thimann, 1957; Geisler, 1963; Geisler, 1967). If CO_2 stimulates intact coleoptiles but inhibits plumule elongation, CO_2 concentration could be part of a mechanism controlling relative rates of growth of coleoptile and plumule and, therefore, the time of plumule emergence.

To test this hypothesis seedlings with coleoptiles 11-16 mm were selected at 48 hr. They were then grown either in the light or dark in distilled water, carbonate buffer or phosphate buffer. The pH of the phosphate buffer

was 6, the same pH as the weaker CO_2 solution. The time of plumule emergence through the coleoptile was recorded for each seedling. In the light, greatest delay of plumule emergence was in seedlings growing on a carbonate-HCl, pH 6 buffer. Seedlings on distilled water showed 50 % emergence at 56 hr i.e. eight hr after transfer to light. Those on phosphate, pH 6 or carbonate, pH 8 buffer reached the 50 % level at 57 hr, while on carbonate-HCl, pH 6 buffer 50 % emergence was reached at 59.5 hr (Fig. 15).

In dark grown seedlings plumules took much longer to emerge than those grown in light. In the dark the first plumules began to emerge at 74 hr i.e. 26 hr after the beginning of treatment. There was no difference in the time of emergence of plumules between seedlings grown in distilled water or the phosphate, pH 6 buffer; 50 % emergence had occurred by 80 hr. On carbonate-HCl, pH 6 buffer, the 50 % level was reached at 84 hr while on carbonate pH 8 buffer, seedlings did not show 50 % plumule emergence until 89 hr.

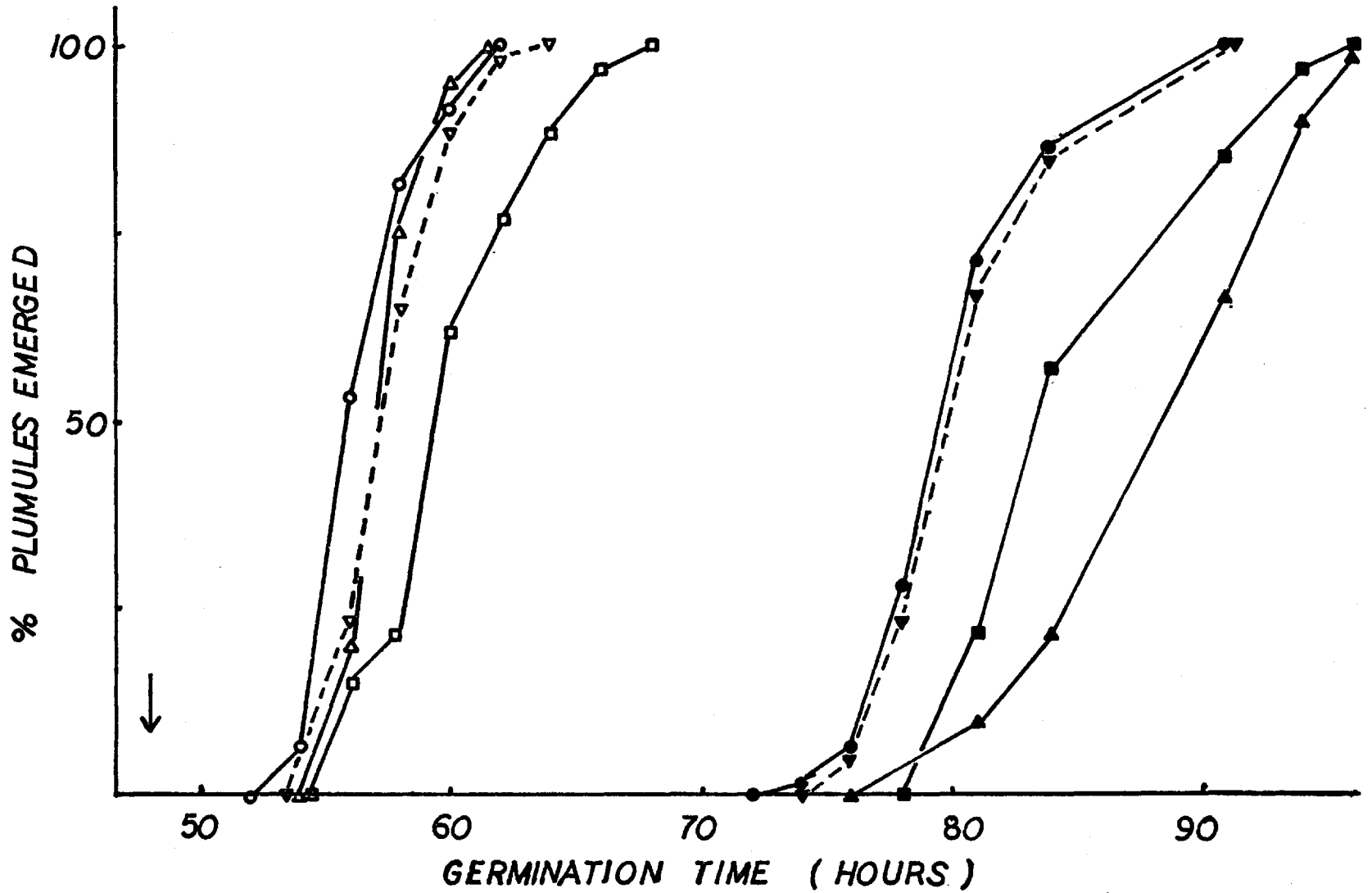
The carbonate-HCl, pH 6 buffer was less effective in delaying plumule emergence in the dark than the 0.1 M carbonate, pH 8 buffer. However, at lower pH levels, CO_2 is lost from the solution, which means that the carbonate-HCl solution must undergo a gradual decrease in CO_2 concentration. This CO_2 loss may account in part for the reduced effect of this solution over long periods of time.

Plumule emergence signifies a change in relative

FIGURE 15

Effect of Various Solutions on the
Time of Plumule Emergence

The percentage of plumules emerged is plotted against time of germination. Beginning of treatments, 48 hr, is designated by the arrow. Seedlings were grown at 25 C in light, open symbols, or dark, closed, from 48 to 96 hr in 12 ml of i) distilled water: o——o , ii) 0.1 M $NaHCO_3$ pH 8.0: △——△ , iii) 0.1 M phosphate pH 6.0: ▽- - -▽ , iv) 0.1 M $NaHCO_3$-HCl pH 6.0: □——□ . Each treatment consists of 60 seedlings with coleoptile heights between 15-20 mm at 48 hr.


100
50
% PLUMULES EMERGED
50
60
70
80
90
GERMINATION TIME (HOURS)

rates of growth of plumule and coleoptile. The plumule emerges because it is growing faster than the coleoptile. Plumule growth was followed in treated seedlings up to 96 hr and the results provide further evidence that CO_2 affects growth. Plumule growth is depressed by high carbonate concentrations. Both in the light and dark plumule height is about 25 % less than in controls (Table XV). The weaker CO_2 solution was less effective in inhibiting growth, but it also reduced growth compared with coleoptiles of seedlings grown on phosphate, pH 6 buffer.

A further test of the ability of CO_2 to influence growth was to incubate segments of coleoptiles and plumules in various solutions for three hr and to measure increase in segment length. It had previously been shown that coleoptile segment elongation was stimulated when CO_2 was bubbled into the water in which they were growing (Rayle and Cleland, 1970). The concentration of CO_2 in such a solution may be near physiological concentrations.

Coleoptile and plumule segments, one five mm segment per seedling and cut four mm below the apex, were taken from class I seedlings of different ages. At 48 hr segments were taken from seedlings with coleoptiles 11-16 mm high, at 72 hr from coleoptiles 26-30 mm high and at 96 hr from coleoptiles 40-60 mm high. There was no stimulation of elongation of 48 hr old coleoptile or plumule segments by CO_2.

TABLE XV

Effect of CO_2 and Low pH on Coleoptile and Plumule Height

Germinating Medium (48-96 hr)	Height (mm) Light			Dark		
	At Time of Plumule Emergence	96 hr	96 hr	At Time of Plumule Emergence	96 hr	96 hr
	Coleoptile	Coleoptile	Plumule	Coleoptile	Coleoptile	Plumule
Water	17.3 ± 2.7	23.8 ± 3.3	53.9 ± 7.8	36.1 ± 6.7	40.6 ± 6.7	59.1 ± 8.2
$NaHCO_3$ 0.1M pH 8	17.0 ± 2.4	23.1 ± 3.1	42.8 ± 5.7	34.6 ± 4.8	37.1 ± 4.9	43.2 ± 7.0
$NaHCO_3$-HCl 0.1M, pH 6	19.1 ± 2.9	24.8 ± 2.9	46.9 ± 5.7	35.9 ± 6.3	40.0 ± 6.9	51.8 ± 8.0
Phosphate 0.1M, pH 6	18.4 ± 3.2	25.2 ± 3.6	55.7 ± 7.7	38.9 ± 5.2	44.6 ± 5.6	63.8 ± 6.8

Sixty seedlings, class I, of uiform height were selected at 48 hr for each treatment. Data are from the same seedlings as in Fig. 15.

The carbonate and phosphate buffer inhibited segment elongation. At 72 hr bubbled CO_2 in water induced a significant stimulation of coleoptile segment elongation and an inhibition of plumule elongation (Table XVI). Again carbonate and phosphate buffers inhibited elongation. At 96 hr coleoptile elongation was stimulated significantly by the CO_2 water solution while plumule growth was stimulated only slightly. The carbonate and phosphate buffers had no obvious effect (Table XVI). Thus, CO_2 stimulates coleoptile elongation but only at 72 and 96 hr. At 48 hr CO_2 inhibits their elongation; with plumules, on the other hand, CO_2 inhibits elongation at 48 and 72 hr and induces a slight stimulation at 96 hr. CO_2 may stimulate or inhibit growth, depending upon the type of coleoptile selected. These results further suggest that in selecting coleoptiles for experimental purposes, it is necessary to bear in mind physiological state as well as height (cf. Section 6).

If a relationship exists between CO_2 respired by the plumule and the growth of the enveloping coleoptile, the CO_2 must be taken up by the coleoptile. Stomate development on the inner epidermis of the coleoptiles was therefore examined. This inhibition of growth by CO_2, at early stages of germination, is examined more carefully in section 15.

TABLE XVI

Effect of Carbonate and Low pH on Coleoptile and Plumule Segment Elongation

Medium	Segment	Average Elongation / Segment (μ/3hr)		
	Age	48	72	96
Water	Coleoptile	300 ± 100c	250 ± 120b	150 ± 130c
	Plumule	520 ± 180a	220 ± 120b	360 ± 100ab
$NaHCO_3$ (0.1M) pH 8	Coleoptile	100 ± 70d	100 ± 90c	160 ± 130c
	Plumule	120 ± 90d	130 ± 100c	320 ± 100b
Bubbled CO_2 in Water	Coleoptile	270 ± 110c	350 ± 190a	320 ± 120b
	Plumule	410 ± 190b	160 ± 100cd	400 ± 80a
Phosphate Buffer 0.1M, pH8	Coleoptile	40 ± 40e	140 ± 90cd	110 ± 100c
		80 ± 80de	150 ± 90cd	310 ± 70b

Coleoptile and plumule segments, see Methods and Materials, were floated on the various solutions for three hr and the increases in individual segment lengths were recorded. Mean increases for each treatment within a germination time suffixed by different letters are significantly different at the 1% level, Duncan's new multiple range test. S.D. are also given.

15. Stomate Development on the Inner Epidermis of the Coleoptile

CO_2 entry into the coleoptile would be facilitated by the presence of stomata. They develop on the coleoptile's inner epidermis and their formation begins before the coleoptile itself begins active growth.

At 15 hr, no coleoptiles have stomata. By 46 hr, however, class I coleoptiles have an average of 62 stomates per coleoptile and this increases to 81 by 68 hr (Table XVII). In class III coleoptiles at 68 hr, the number of stomata on the inner surface ranges from 0-46. This group of coleoptiles includes IIIa, IIIb and IIIc types and by 75 hr there is a clear separation of these types both on the basis of height and number of stomata. At 68 hr all class III coleoptiles are similar in height. Those of class IIIa, however, could probably be recognized at 68 hr by counting stomata number. Since at 75 hr their mean stomate number is 0.3 and the range is 0-2 (Table XVII) they must constitute the 68 hr coleoptiles with 0 stomates. The presence at 68 hr of class III coleoptiles with 46 stomata shows that, like class I coleoptiles, stomata formation precedes the onset of active growth. It is also apparent that formation of stomata continues after the onset of active growth and class IIIc and IIIb coleoptiles form more stomata per mm

TABLE XVII

Number of Stomates on the Inner Epidermis of Coleoptiles of Different Classes

Germination Time (hr)	Coleoptile Class	Height (mm)	Stomate Number	Range
15	A	2.6 ± 0.6	-	-
46	I	12.5 ± 1.0	62 ± 7.9	48-74
68	I	36.0 ± 2.9	81 ± 7.3	66-92
68	III	8.0 ± 1.6	17 ±15.4	0-46
75	IIIa	5.3 ± 1.1	0.3± 0.7	0-2
75	IIIb	10.6 ± 1.9	53 ±17.8	29-82
75	IIIc	20.5 ± 2.6	136 ±16.5	114-157
115	IIIa	6.3 ± 2.5	19 ±20.9	0-49
115	IIIb	21.3 ± 4.6	94 ±21.8	47-123
115	IIIc	50.7 ± 3.1	131 ±24.4	107-154

Ten coleoptiles were used for each determination. A, refers to the tallest coleoptiles at 15 hr of germination.

coleoptile than class I. At 75 hr, IIIc coleoptiles have 136 ± 16 stomata while class I coleoptiles at 68 hr have only 81 ± 7.3 stomata, even though the latter coleoptiles are almost twice as long. Thus, though stomates appear at different times in different coleoptiles, their development seems to be associated with active growth. It may be a critical aspect of growth that stomata develop on the inner epidermis in coleoptiles that are growing actively. This result and the fact that coleoptiles that fail to grow have few stomata support the suggestion that CO_2 from the plumule plays a part in regulating growth.

16. Effect of CO_2 on Coleoptiles in Early Stages of Growth

In section 14, it was shown that CO_2 inhibits coleoptile and plumule elongation in the early stages of germination. To test the effect of CO_2 on slowly growing seedlings, early in their development, a group was selected at 24 hr. These seedlings had no roots; only the coleorhiza had emerged. Seed coats were removed from half of the seedlings. They were then grown in distilled water or 0.1 M $NaHCO_3$, pH 8 buffer.

Removal of the seed coat increases the frequency of tall coleoptiles and reduces that of short coleoptiles; there are 40 % more coleoptiles greater than 20 mm high

when coats are removed than when they are intact (Table XVIII). All coleoptiles are not affected by this treatment, however, since at least 5 % are less than 10 mm high at 68 hr. Mean height of coleoptiles from seedlings with coats removed is 20.6 mm and 15.0 mm in controls (Table XVIII).

Seedlings grown in a 0.1 M $NaHCO_3$ pH 8 buffer do not have any coleoptiles greater than 20 mm in height; the average height of coleoptiles of seedlings with intact coats and in the carbonate buffer is 8.5 mm. This is less than of seedlings with their coats removed and in the carbonate buffer, 10.9 mm (Table XVIII). Coleoptiles from seedlings with intact seed coats and germinated in distilled water are 27.2 % shorter than if the coats are removed. Seedlings with their coats intact and germinated in a carbonate solution are 31.6 % shorter than those germinated in distilled water. CO_2, therefore, is either delaying the initiation of rapid growth in some seedlings or is inhibiting growth of all seedlings.

CO_2, thus, has a strong influence on coleoptile and plumule growth rates throughout germination. During the early periods of germination high CO_2 concentrations inhibit both coleoptile and plumule elongation. At a particular time in germination i.e. between 44-72 hr of germination, the CO_2 effect on coleoptile elongation is reversed; elon-

TABLE XVIII

Effect of Seed Coat Removal and CO_2 on Seedling Growth Early in Germination

Seed Coat	Growth Medium (24-68 hr)	Coleoptile Height (mm)	Percent Distribution at 68 hr.		
			1-9mm	10-20mm	over 20mm
Intact	Water	15.0 ± 6.5	20.0	56.7	23.3
Intact	0.1M $NaHCO_3$ pH 8.0	8.5 ± 4.0	46.7	53.3	
Removed	Water	20.6 ± 5.0	5.0	31.7	63.3
Removed	0.1M $NaHCO_3$ pH 8.0	10.9 ± 2.4	51.7	48.3	

One hundred and fifty seedlings having only the coleorhiza emerged at 24 hr were used for each treatment. Coleoptile heights were measured at 68 hr.

gation is then stimulated. Plumule elongation, however, is still inhibited.

CO_2 is exerting its inhibitory effect at times when cells in these organs are dividing actively and rates of cell expansion are increasing. Since these processes require high metabolic rates CO_2 may be inhibiting growth by affecting respiration. In some respects, the effects of CO_2 on coleoptile growth mimic the effects of kinetin and gibberellin, i.e. growth is inhibited early in germination and stimulated at some later times. With each compound, however, growth is not initiated but rather is modulated.

17. Inheritance of Genetic Growth Patterns

The variation observed in the pattern of growth of coleoptiles means, in terms of coleoptile growth, this population shows phenotypic polymorphism. This could be due to an underlying genetic polymorphism, in which case a high degree of correlation between growth patterns of a plant and its offspring could be expected. To determine whether there is a genetic factor responsible for the patterns of coleoptile growth, seedlings with the various classes of coleoptiles were grown to maturity and their seeds were collected and germinated.

One class I seedling was studied. Its 20 progeny

included one class I, three class II and 16 class III types. These plants were grown to maturity and their seeds were germinated in the normal way. The class I plant gave poor seed set, but the class II and III plants produced many viable seeds. Both of them gave rise to class I, II and III types (Table XIX).

In the case of the 16 class III seedlings though the parents grew slowly in the first 70 hr, 99 out of 212 offspring were class I. The percent distribution of seedling types is very similar to that from a random sample of seeds (Table XX). The random sample of seeds consists of 45.6 % class I, 32.0 % class II and 22.1 % class III seedlings. Barley plants that were class III during their germination produced seeds with a distribution of 46.7 % class I, 33.0 % class II and 20.3 % class III (Table XX). It appears, therefore, that there is no correlation between growth pattern of parent and progeny. When a large sample of seedlings is taken, distribution of coleoptile classes is the same from either a random sample or from plants whose parents were class III. These results provide no evidence of a genetic component determining pattern of seed germination and coleoptile growth.

Not only do class III seedlings produce the same percentage distribution of seedling types but final plant heights are very similar to those of class I (Table XXI).

TABLE XIX

Type of Progeny to the Third Generation from a Class I Seedling

Class of Parent	Number of Plants	Progeny (No. of Seedlings) Class I	II	III
		Generation 2		
I	1	1	3	16
		Generation 3		
I	1	0	1	0
II	3	17	15	21
III	16	99	70	43

TABLE XX

Progeny from Class III and a Random Sample of Seeds

Parent	No. of Seedlings	Percent Distribution of Progeny		
		Class I	II	III
Random	823	45.6	32.0	22.1
III	212	46.7	33.0	20.3

TABLE XXI

Final Plant Heights of Class I and IIIa Seedlings

Class	No. Plants Observed	Final Plant Height (cm)	Range (cm)
I	76	46.0 ± 4.9	34-55
IIIa	12	46.5 ± 3.9	38-51

Heights were measured at maturity. S.D. are given for each average height.

Class I seedlings have an average final plant height of 46.5 cm. The two types of plants grow differently as seedlings early in germination, but their final plant heights are alike.

There does not appear to be any correlation between the pattern of germination of a seedling, its final height as a mature plant, and the growth patterns of coleoptiles of the seeds it produces. The results suggest that the segregation of coleoptiles, on the basis of growth pattern, is not under genetic control. Furthermore, since a random sample of seeds is taken from a population of plants that grew under essentially the same external environmental conditions, it does not appear that external physical conditions during seed formation are responsible for the various classes of coleoptiles.

During seed formation, embryos do not grow under identical internal conditions. Preliminary data showed that individual seeds from one barley plant do not all have the same pattern of germination. Furthermore there seemed to be no correlation between position of seed on the spike and germination pattern. It is probable then that different embryos reach different stages of development before they become dormant. These differences may be expressed in germination in different patterns of growth.

DISCUSSION

1. Variation in Coleoptile Growth Patterns

Variation in the time and rate of maximum growth of individual coleoptiles is an intrinsic trait of germinating barley seeds (Fig. 6,7). Of a sample of seeds set to germinate at one time, some coleoptiles grow tall, others are medium and a few remain short in height by 68 hr. Moreover, some of the short and medium coleoptiles increase their rate of growth after 68 hr to become tall, whereas, others stop growing and remain short in height. Thus, seedlings from this inbred population of barley are rather heterogenous in their patterns of growth even though their final heights are similar. In analysing growth patterns of coleoptiles the first step was to standardize external conditions, i.e. to reduce variation in physical conditions to a minimum and then to determine whether a change in external environment affects the variability in coleoptile growth.

2. Water Volumes for Germination: Effect on Coleoptile Growth

Physical conditions under which seeds germinate may

affect the extent of coleoptile growth. Even slight increases in water volumes above the optimum for growth, reduces coleoptile elongation in the first 72 hr of germination. Percentage germination is also reduced as the water volumes are increased. Dasberg and Mendel (1971) obtained similar results for germination of Triticum vulgare and Oryzopsis holciformis seeds in petri dishes containing various amounts of water. These workers suggest that the decrease in rate and percentage of germination, under conditions of excess water, is caused by "impaired diffusion of oxyygen to the germinating seed". The results show that the coleoptile growth patterns are determined internally; they are not controlled by physical conditions.

3. Variability in Auxin Bioassays

Even if physical conditions are kept constant, however, variation in growth from one coleoptile to another is present. It appears that earlier workers were not aware of the variation in coleoptile growth and they used coleoptile segment elongation in auxin bioassays. There has always been remarkable variability from one experiment to another with the bioassay (Went and Thimann, 1937; Rayle et al.,1970). The criteria previously used for selecting test coleoptiles were height and age, and not growth rate (McCrae and Bonner,

1953; Nitsch and Nitsch, 1956). The results presented here show that only those segments from actively growing coleoptiles respond to IAA in every trial. Samples of coleoptiles that were uniform in height, but mixed in their growth rates showed variable responses to IAA. Some responded while others did not. IAA does not induce elongation in segments from coleoptiles that were not growing actively at the time they were selected. Furthermore, IAA supplied to the seedling via the roots also fails to stimulate slowly growing coleoptiles to elongate rapidly, in fact all coleoptiles are somewhat retarded by this treatment. Chadwick and Burg (1967) suggest that coleoptile growth is inhibited indirectly because of the inhibition of root growth by IAA. Thus, IAA cannot initiate elongation in coleoptiles that are not growing actively, but can increase elongation in segments from rapidly growing coleoptiles. This effect is similar to that of kinetin and gibberellin (section 5). The variable response to IAA shows that morphologically similar coleoptiles may be physiologically different.

4. Coleoptile Cell Number

Physiological variability within morphologically similar coleoptiles is also shown by counts of cell number. During the main period of growth the extent of cell prolifer-

ation is not equal in all coleoptiles. The shorter coleoptiles are relatively less efficient in undergoing cell division than cell elongation. This is borne out when when class IIa and IIb or IIIb and IIIc coleoptiles are compared.

Class IIIa coleoptiles are unique; though during seed formation, in embryogenesis, they develop the same number of cells as the others, during germination they undergo little or no cell division and show only a ninefold increase in height. The growth in both aspects is either repressed or cannot be initiated. Yet the lack of growth is limited to the coleoptile; leaves eventually reach a normal final height. The fact that cell number is apparently the same in all embryonic coleoptiles emphasizes that the differences found after 96 hr of germination are due to differential growth, cell proliferation and elongation, in different coleoptiles. Therefore, whatever regulates coleoptile growth is expressed to different degrees in different coleoptiles.

One factor may be the ability to intiate DNA synthesis. In the wheat embryo, for example, a period of protein synthesis prior to nine hr of germination is required for the onset of DNA synthesis and cell division (Mory et al., 1972). However, even after irradiation which completely suppresses cell division, some coleoptiles can initiate rapid growth. Moreover, the variation in pattern of growth among individual coleoptiles is the same whether seeds are

irradiated or not. Thus, it appears that the stimulus for active growth is separate from the mechanism that intiates cell division. Coleoptiles can grow to 32 mm without cell division i.e. by abnormally high amounts of cell expansion.

5. Plant Growth Factors

Even though kinetin and gibberellin have been implicated in the control of cell division and cell elongation exogenous applications to germinating barley seeds did not initiate active growth of those coleoptiles that were growing slowly. Though a number of growth processes are stimulated by kinetin (Guttman, 1956; Miller, 1961), it is clear that barley coleoptiles fail to respond to kinetin at a specific stage of their development. Whether in terms of cell division or cell elongation (Wright, 1961; Burrows and Carr, 1970; Sveshnikova and Khokhlova, 1969), the actual mechanism of the kinetin effect is still unknown.

However, unlike the results of Wright (1961), the studies presented here, on coleoptiles selected both for height and growth rate, show that they respond to kinetin at the time when most of the increase in cell number has been completed i.e. from 34-44 hr in class I coleoptiles. Furthermore, cells are in a specific stage of expansion i.e. active growth has been initiated and cells are increasing

their rate of elongation rapidly. Before this time kinetin has no, or an inhibitory effect on coleoptile growth. The apparent conflict in the time of kinetin's effect on coleoptiles between the observations of Wright (1961) and those presented here, may in fact be the result of variation in rate of growth of individual coleoptiles used by Wright.

Seedling germination consists of a series of sequential processes viz. 1) imbibition of water by the seed, 2) activation of embryo growth, 3) development of vascular connections between endosperm reserves and the embryo and, 4) mobilization and transport of endosperm reserves to the developing embryo. Functions in the initiation and continuation of these processes have been ascribed to a number of the growth factors. In particular, it has been suggested that gibberellin initiates barley endosperm reserve degradation (Varner and Johri, 1968) and the initial growth of the embryo (Kahn, 1971). The suggestions imply that a) endosperm reserves are important to the growth of the embryo at very early stages in germination and b) that there is a transport route, connecting the embryo and the endosperm for movement of gibberellin to, and degraded reserves away from the endosperm in the early stages of germination.

However, barley endosperms do not appear to be degraded until about two days of germination (James, 1940; James and James, 1940). Furthermore, work with pine seed-

lings suggests endosperm reserves are not necessary for the initial growth of the embryo (Sasaki and Kozlowski, 1969). Work on the wheat scutellar vascular system shows it is completely undifferentiated in the dry seed and only after about a day of germination is there a transport route connecting the embryo and the endosperm (Swift and O'Brien, 1970; ibid, 1971). In addition, studies on localization of hydrolases in germinating wheat seeds suggest that storage reserves of the coleorhiza rather than those of the endosperm are important to embryo growth early in germination (Price and Ey, 1970). Moreover, though gibberellin has been reported to initiate degradation of barley endosperm halves by Paleg (1961) and Varner (1964) neither of these workers indicated when in germination this happens. In contrast, MacLeod and Millar (1962) suggested gibberellin does not initiate endosperm reserve degradation but rather stimulates it once it has been initiated by some other means. Further support for this suggestion comes from work that shows that aspartate, glutamate or organic acids are also able to stimulate endosperm degradation(Galsky and Lippincott, 1971; Tamura et al., 1967).

Thus, the stimulation of coleoptile elongation by gibberellin is approximately coincident i.e. 44 hr onwards (Table XI), with endosperm reserve degradation (James, 1940; James and James, 1940) and may result from a rise in the

level of available metabolites. It is suggested, therefore, that gibberellin promotes endosperm reserve degradation at the same time that it stimulates coleoptile growth. In this capacity, gibberellin would not initiate physiological processes, but rather promote those that have already been initiated. Stuart and Cathey (1961) in their review on gibberellin arrive at the same conclusion i.e. "for the most part, applied gibberellin does not establish new growth patterns but accelerates those in progress."

Thus, in barley coleoptiles none of the growth factors, gibberellin, kinetin or auxin appear to be able to initiate active growth. In all cases, in the studies presented here, the three appear to modulate growth that had previously been initiated by some other means. Each appears to be able to promote growth only when the plant is in a particular i.e. a physiologically sensitive state. If this physiological state has not been achieved, then the growth factor cannot promote growth and may, in fact, inhibit it.

6. CO_2 Effects on Coleoptile Growth

The response of coleoptiles to CO_2 is somewhat comparable to their response to the growth factors. At one time in germination both inhibit growth while at another time they stimulate it. A similarity of site of action is

not implied by similarity in the response they evoke. At about the time when CO_2 inhibits growth, coleoptiles are accumulating dry matter. Since this process requires a high rate of metabolism, CO_2 could be inhibiting growth at this time by inhibiting respiration (see Beevers, 1961). Moreover seed coats may lead to a build up of CO_2, in the beginning of germination, since removal of seed coats results in greater rates of growth both in barley coleoptiles (Table XVIII) and in peanut embryos (Toole et al., 1964).

At later times CO_2 stimulates coleoptile extension. However, it is clear that CO_2 is only one of many factors eg. IAA, gibberellin or inositol that can stimulate growth of the coleoptile. By choosing coleoptiles at a sensitive stage one can show stimulation by a number of compounds. Growth, therefore, is not regulated by one compound alone, eg. CO_2, but by many factors.

7. Coleoptile Protein and Free Amino Acids

Both the alcohol soluble and insoluble amino acids can differ among individual coleoptiles. A striking feature only of those coleoptiles that initiate active growth is the increase in protein levels and in particular the change in the level and percentage composition of the free amino acids. Free amino acids generally increase substantially in germ-

inating seedlings (Fowden, 1967; Wang, 1969). One or more of the amino acids may increase to levels much higher than others. For example, asparagine is the predominant free amino acid in wheat shoots, and proline in corn (Wang, 1969). "Shoot" in these cases probably means coleoptile and plumule. In barley coleoptiles serine increases in amount and constitutes over 60% of the total free amino acids by 85 hr (Table XI). While serine increases, glutamate-glutamine and alanine decrease in the alcohol soluble fraction. Glutamate is known to be a nitrogen source for serine biosynthesis in pea seedlings (Slaughter and Davies, 1969); alanine could also be contributing nitrogen for serine biosynthesis. Thus, the increase in serine levels during germination could be related to the decrease in glutamate-glutamine and alanine.

Accumulation of one amino acid, serine, to such high amounts must be for specific reasons. Serine has been shown to be in metabolic proximity to photosynthesis (Hellebust and Bidwell, 1963). It follows then that plumules should be capable of metabolizing serine quite readily. It is suggested, therefore, that serine in the coleoptile may be a reservoir for the plumule; it could be transported from the coleoptile and metabolized by the plumule as it emerges. Serine would be promoting plumule growth while the plumule's photosynthetic machinery was

developing to its full capacity.

Only those coleoptiles that show active growth have major changes in levels and compositions of free amino acids and proteins. Protein levels, though only one of many possible indicators of gene activity should provide evidence on the type of control that regulates coleoptile growth. Though changes in protein level do not show why some coleoptiles are more active in growth than others they suggest there might be a genetic component involved in determing patterns of growth.

8. Inheritance of Coleoptile Growth Patterns

Genetic factors, however, do not seem to determine the pattern of growth of coleoptiles during germination. Since no evidence has been found of a genetic factor influencing the pattern of coleoptile growth this regulatory function has been attributed to cytoplasmic constituents. Hayward and Lawrence (1970) also could not find a genetic component controlling rate of germination in the forage grass, Lolium perenne. It is suggested, therefore, that the pattern of growth of a coleoptile is pre-determined during seed formation. The fact that the seeds from one plant (see Results, section 16) have coleoptiles with different growth patterns further indicates that this variation results from the internal conditions of individual

seeds rather than from the external environment. It is suggested that 1) the nature of the internal conditions controlling growth patterns is cytoplasmic and 2) cytoplasmic differences arise by unequal divisions of the zygote during early embryogenesis.

Norstog (1972) showed that in barley embryos, in very early stages of development, there are substantial differences in cell size within one embryo and in cells in comparable positions in different embryos. Furthermore, he reports that cell divisions at the five-celled stage "are not always regular and cell walls are disposed at various angles to the presumptive embryonic axis." In developing carrot seeds, Borthwick (1931) showed that 1) the sequence in which cells divide is extremely variable among individuals of the same species and 2) the various parts of the mature embryo are not always derived from the same cells of the four-celled embryo. For example, the distal cell may give rise to only the cotyledons and upper part of the hypocotyl, the cell next to it to the rest of the embryo while the remaining two cells form the suspensor. In another case, the entire embryo may be derived from the distal cell and the suspensor from the remaining three cells. Inequality of division of cytoplasm will result in a distribution of cytoplasmic constituents that is variable from one embryo to another.

Thus comparable parts of embryos, for example the coleoptile will differ in cytoplasmic constitution. Such differences are known to influence gene expression and it is suggested, therefore, that the differences in coleoptile growth patterns is a consequence of cytoplasmic variability among coleoptile primordia. This inherent variability in growth patterns is revealed only when the detached seeds are no longer assisted by the resources of the parent plant. Differences in cytoplasm content of developing embryos might also account for the lack of correlation during germination in growth of the barley root and coleoptile i.e. the roots of a seedling may grow actively though the coleoptile grows slowly (Table X, XI).

The variation in growth among individuals of inbred organisms has also been seen in all other plant species examined i.e. corn, wheat, rye oats and squash. Furthermore, variation in growth of embryos, even among highly inbred organisms, is not confined to plants. Williams et al (1962) reported significant variations in growth, and in a number of biochemical parameters, among individual animals from inbred lines.

Individuals from inbred populations have generally been selected for homozygosity at a limited number of loci. At other loci there could be substantial heterozygosity remaining in the population. Yet in inbreeding and out-

breeding lines there is variation in growth. There are also significant differences in growth of monozygous armadillo quadruplets in which the genotypes are identical. The variations are apparently the result of unequal divisions of the cytoplasm of the zygote in early embryogenesis (Storrs and Williams, 1968).

Cytoplasmic variability because of unequal divisions in the zygote is clearly, therefore, a factor contributing to different growth patterns among individuals having similar or identical genotypes. In barley, this variability is temporary and can be overcome since the plant eventually grows tall even though its growth is retarded at the stage of its development when the coleoptile is undergoing its major period of growth.

CONCLUSIONS

A population of coleoptiles is not uniform in height during germination. Differences in height exist because all coleoptiles do not grow uniformly; rather, they follow one of six growth patterns. The patterns result from differences in the time of initiation and extent of active growth of individual coleoptiles.

The variability in growth has implications with respect to selection of coleoptiles as experimental material. It is suggested that variability in results using coleoptile elongation as a test system should be attributed to selection of coleoptiles that are morphologically similar viz. in height, but are physiologically different i.e. in growth rate.

Lack of the growth factors kinetin, gibberellin, or IAA in some coleoptiles does not appear to be the cause for variation in their growth since exogenous application of these growth factors does not induce active growth in slowly growing coleoptiles. It is concluded, therefore, that in the period when they stimulate growth these compounds are modulators of growth processes that are already operating.

Though even slight changes in physical conditions

affect growth, variation in growth is found under any external conditions. This means that variation results from differences in internal factors among individual coleoptiles.

The changes in composition and levels of proteins occurring with initiation of rapid elongation indicate the differences in growth among coleoptiles has a basis in differences in expression and activity of genes.

Finally, the variation does not appear to be genetically controlled. It is concluded that cytoplasmic differences arising among individuals at the time of coleoptile primordia formation during embryogenesis, are responsible for the variation in growth among coleoptiles.

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APPENDIX

1. Test for Skewness in 66 Hr Coleoptile Height Distribution

$$\text{Statistic } \sqrt{b_1} = m_3/m_2^{3/2} \quad \text{where } m_r = \frac{\sum_{i=1}^{n} (X_i - \bar{X})^r}{n},$$

$$r \geq 2$$

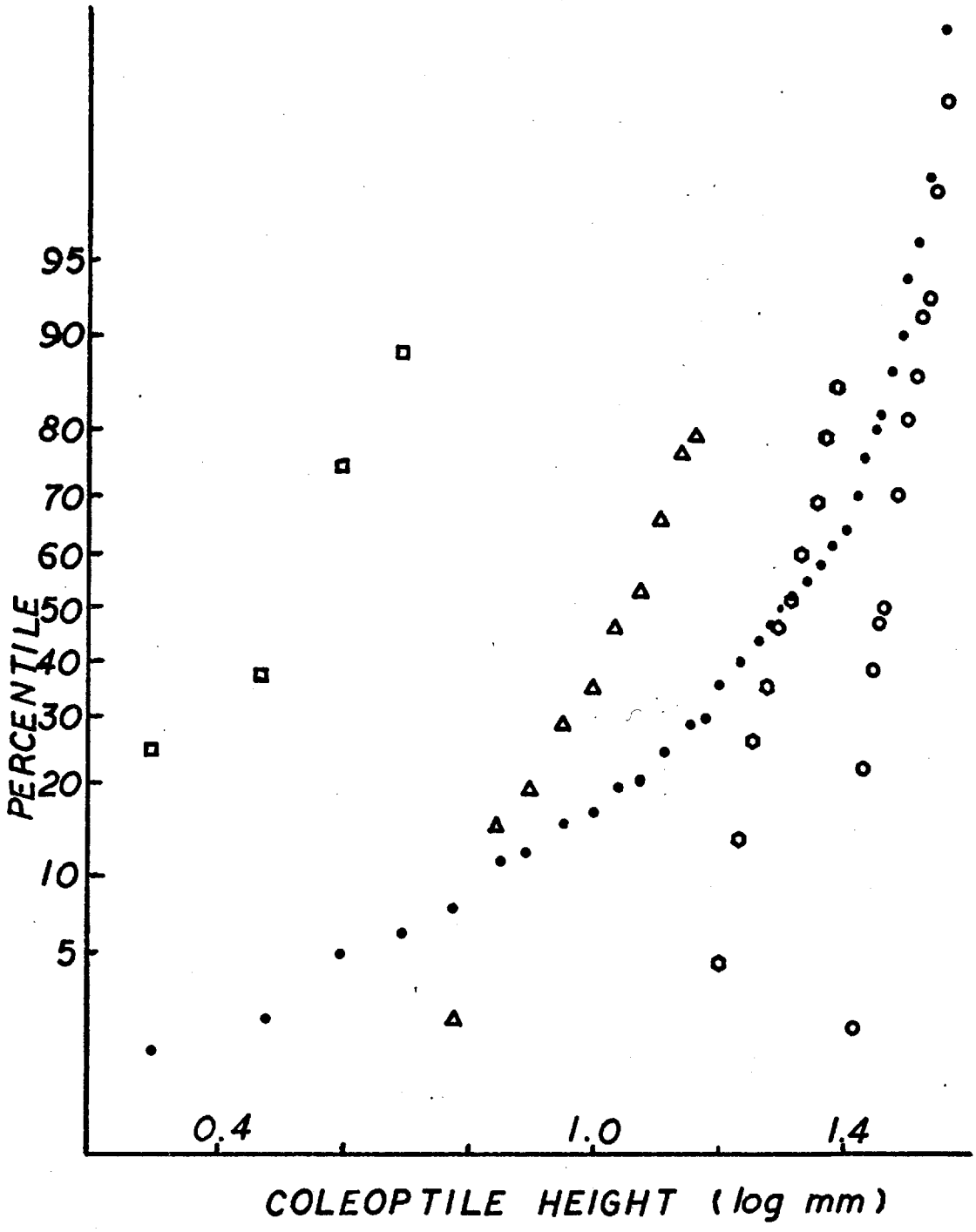
$\sqrt{b_1}$	Observed	Expected
	0.291	0.202 (1 %)
		0.142 (5 %)

Therefore distribution is skewed.

FIGURE 16

Probit Analysis of 66 Hr Coleoptile Height Distribution

Percentile of coleoptile heights at 66 hr of germination is plotted against the log of the coleoptile height, •••• . From the approximately linear phases between the points of inflection of the original curve, four subpopulations are evident i.e. A, 2-5 mm, ▫ ▫ ▫ ; B, 6-15 mm, △ △ △ ; C, 16-25 mm, ○ ○ ○ ; and D, 26-37 mm ○ ○ ○ . The data are based on 821 coleoptile heights.


95
90
80
70
60
50
40
30
20
10
5
PERCENTILE
0.4
1.0
1.4
COLEOPTILE HEIGHT (log mm)

2. Test for Significant Differences among Subpopulation Means of 66 Hr Coleoptile Height Distribution

Subpopulation	Height Range (mm)	Mean Ht (log mm)	Sample Size	S.D.
A	2-5	0.48	47	0.180
B	6-15	1.05	197	0.145
C	16-25	1.32	279	0.145
D	26-37	1.47	291	0.035

Subpopulation Comparisons	T Observed	T Expected (5 %)	DF
A-B	22.95	1.60	242
A-C	35.28	"	324
A-D	84.91	"	342
B-C	19.97	"	474
B-D	47.76	"	492
C-D	17.27	"	574