# ON THE RELATION BETWEEN GROWTH AND RESPIRATION IN THE AVENA COLEOPTILE

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## I

## INTRODUCTION

The discovery of the auxins and their activity in controlling plant growth has raised certain questions of a novel type. Of these perhaps the most fundamental is that of the mechanism of the action: namely, of how a single substance can bring about the elaborate series of changes that comprise growth. Attempts at a solution of this problem have been made along the following three lines: (1) study of the chemical nature of the auxins; (2) study of the mechanical changes, particularly those of the cell wall, which accompany growth; and (3) analysis of the intermediate stages between the supplying of the hormone and the first appearance of the growth phenomena. The results of these experiments have been discussed by Went and Thimann (1937) and by Thimann and Bonner (1938) and need not be considered as a whole here. The essential data pertinent to the present research are as follows:

A close connection exists between growth and respiration. At least in the *Avena* coleoptile, growth will not take place without respiration; and if respiration is partially reduced by treatment with the proper concentration of cyanide, growth is reduced to the same extent (Bonner, 1936).

A close connection also exists between growth and protoplasmic streaming. Those concentrations of auxin which accelerate growth also accelerate the rate of streaming, and the effect on streaming is observable long before any effect on growth can be detected (Thimann and Sweeney, 1937). For both growth and streaming sugar plays an essential rôle as an accessory substance; it increases the amount of growth resulting from a given auxin concentration (Schneider, 1938), and it prolongs the acceleration of streaming caused by auxin (Sweeney and Thimann, 1938).

The effect of various respiratory substrates on growth also serves to indicate an interrelation between growth and respiration. Thus, sugar is essential to the growth of isolated coleoptile sections, and the four-carbon

acids, malic and fumaric, when supplied together with auxin and sugar further increase the growth of such sections. This latter finding, which was observed in certain preliminary experiments, served as a guide to the work to be discussed, and it will be shown that this effect sheds a good deal of light on the relation between auxin and respiration.

All these data point in the direction of close interrelation between the processes of growth and respiration. Several attempts have been made to demonstrate some respiratory effect of auxin. However, in all cases the addition of auxin (in concentrations which accelerate growth) had no effect on the rate of respiration of *Avena* coleoptiles (Bonner, 1936;[1] Van Hulssen, 1934). It was apparent therefore, that the problem called for an examination in greater detail.

The present paper represents a study of the relation between growth and respiration in the *Avena* coleoptile, and an attempt to analyze the physiological basis of the effects of auxin.

## II

## *Materials and Methods*

*Avena* coleoptiles were grown in the usual way on filter paper in a dark room at 24°C. and 85 per cent relative humidity, with occasional red light. The plants were used at the age of 76 hours from the time of soaking unless otherwise stated.

As auxin, pure indole-3-acetic acid was used throughout. The malic acid used was decolorized and recrystallized from a C.P. sample. All solutions were made up freshly once a week. Acid solutions were neutralized to pH 6.8 with KOH.

Measurements of growth were made on isolated sections of the coleoptile 3 mm. long placed on combs floating on the surface of the solution used (as described by Schneider, 1938). Thirty sections obtained from ten coleoptiles were used in each such experiment. Respiration was measured either in Warburg manometers using thirty sections or in the microrespirometer previously described by Thimann and Commoner (1940) using only a single section. In some cases the sections used for respiration experiments were measured for length by direct examination with a calibrated microscope (at the end of the run). The growth measurements were carried out in the dark moist room at 24° and the respirometer water baths were held at the same temperature and shielded from light.

## III

## EXPERIMENTAL

The fact that growth is dependent upon the presence of oxygen and that both growth and respiration are proportionally inhibited by cyanide, provides a clue to the nature of the link between these processes.

[1] In earlier work Bonner (1934) reported that growth substance stimulated the respiration of *Avena* coleoptiles. He later concluded that this effect was due to impurities in the preparation since subsequent measurements with pure crystalline auxin had no effect (1936).

The two end substances involved in respiration are oxygen and the substrate which is oxidized (*e.g.*, sugar). In many plant and animal cells the Warburg-Keilin respiratory system appears to mediate 90 per cent of the oxidation. According to this scheme, molecular oxygen is activated by an oxidase enzyme, and the metabolite is prepared for oxidation by the system of dehydrogenases specific to it. Intermediate carriers link these two ultimate enzymes. Thus:

$$\text{Oxygen} \rightarrow \text{oxidase} \leftrightarrows \text{carriers} \leftrightarrows \text{dehydrogenase} \leftarrow \text{metabolite}$$

Cyanide poisons the (cytochrome) oxidase and thereby prevents the utilization of molecular oxygen. Since the reduction of respiration caused by various concentrations of cyanide is always accompanied by a proportional decrease in growth, it is indicated that the oxidase is necessary for *both* processes. On the other hand we know that respiration may take place without any accompanying growth, so that the same *dehydrogenases* cannot be necessary factors for both processes. It follows that if any separation between growth and respiration is to be experimentally effected, this must occur at the dehydrogenase end of the respiratory sequence.

It was therefore decided to test the effect of various known inhibitors and accelerators of dehydrogenase activity upon the growth rate.

### *1. The Effect of Dehydrogenase-Active Substances*

Growth measurements were made on sections of the *Avena* coleoptile, using the method described above. Combs carrying thirty sections were floated on solutions made up to a concentration of 1 per cent sucrose and 1 mg. per liter of indole-3-acetic acid. To this solution were added various dehydrogenase-active substances and growth measurements made over a period of about 48 hours.

Table I gives the relative growth in 24 hours of sections floated on the various solutions. From this it can be seen that the complete activity of the various dehydrogenases is necessary for complete growth. Urethane, which is a general inhibitor of dehydrogenases, causes a noticeable diminution of growth. Pyrophosphate, which is known to inhibit the succinic dehydrogenase, has a more marked effect. Malonic acid, another inhibitor of succinic dehydrogenase, has a similar effect on growth; while barbital, which is structurally related to malonic acid, exerts a more marked inhibition.

The most striking reduction in growth was obtained with (mono)-iodoacetic acid. This effect which was noticed in passing by Bonner (unpublished) has been recently reported also by Howard and McClintock (1940). Our experiments were carried out independently of those of How-

ard and McClintock but in so far as they are comparable our results do not disagree with those obtained by them.

Iodoacetic acid is known to inhibit a number of dehydrogenases and related enzymes. Among those enzymes affected are glyoxalase, "apozymase," lactic dehydrogenase, malic dehydrogenase, $\beta$-hydroxybutyric dehydrogenase, alcohol dehydrogenase, $\alpha$-glycerophosphate dehydrogenase, and phosphorylating enzymes (*cf.* Cohen, 1939). The effect of iodoacetic acid on these enzymes seems greatly to depend on the concentration of the poison and on the state of the enzyme (*i.e.* whether *in vivo, in vitro, etc.*) and in some cases is in dispute. However, in the present case, as the data presented below demonstrate, the iodoacetic acid effect seems to be rather specific.

TABLE I

| Substance | Concentration | Relative growth in 24 hrs. |
|---|---|---|
| Sucrose | 1 per cent | 100 |
| Auxin | 1 mg./l. | |
| Sucrose alone | 1 per cent | 54 |
| Iodoacetic acid + sucrose + auxin* | 0.01 M | −12 |
| Malonic acid + sucrose + auxin | 0.01 M | 39 |
| Barbital + sucrose + auxin | 0.01 M | 7 |
| Urethane + sucrose + auxin | 0.01 M | 58 |
| Pyrophosphate + sucrose + auxin | 0.01 M | 33 |

* These solutions all include 1 per cent sucrose and 1 mg. of auxin per liter.

The effect exerted on growth by various metabolites which would be expected to be dehydrogenated by the coleoptiles is shown in Fig. 1. Sucrose, of course, increases the growth of sections in auxin (Schneider, 1938). In the presence of sucrose and auxin together, succinate, malate, fumarate, and pyruvate all increase the growth rate over that in sucrose and auxin alone. The acceleration of growth by the four-carbon dicarboxylic acids (and pyruvic acid) occurs only after the first day of growth.

It is clear, therefore, that certain inhibitors (iodoacetate in particular) which are known to reduce the respiratory activity of various dehydrogenases, are also inhibitors of growth. Further, the four-carbon acids, and pyruvic acid, which are known to constitute an important reversibly oxidizable chain in the sequential processes of cell respiration (Szent-Györgi, 1935) accelerate the growth rate of coleoptile sections.

### *2. The Effect of Iodoacetate on Growth and Respiration*

In the light of the above results it seemed important to examine in greater detail the effect of iodoacetate. Growth measurements were made in the

usual manner, the sections being exposed to solutions containing 1 per cent sucrose, 1 mg. of auxin per liter, and various concentrations of K iodoacetate. The results are shown in Figs. 2 and 3.

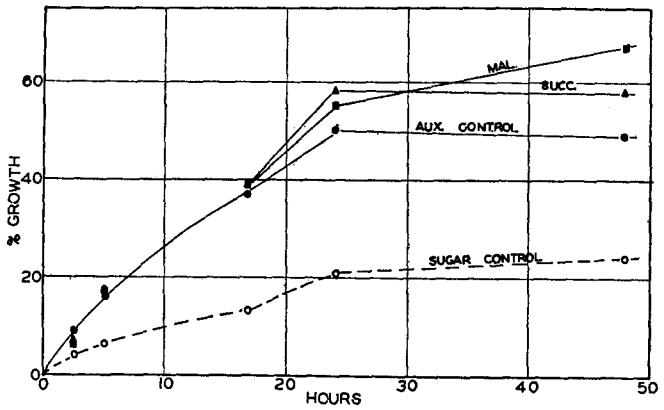


FIG. 1. The effect of malate (0.001 M) and succinate (0.001 M) on the time course of growth. All solutions contained 1 per cent sucrose and all but the sugar control contained 1 mg. of auxin per liter.

These data demonstrate that the effect of iodoacetate on growth is greatly dependent on concentration. Concentrations of $10^{-6}$ or $10^{-5}$ M actually accelerate the growth rate, while concentrations of $2 \times 10^{-5}$ or greater produce a marked inhibition which becomes complete at $5 \times 10^{-5}$ M. The shape of the growth curve in Fig. 3 is characteristic of the effect of many poisons and other active substances on enzymatic processes. Thus cyanide and carbon monoxide in low concentrations sometimes accelerate oxygen consumption, and the effect of auxin itself on growth follows a similar curve (Thimann, 1937).

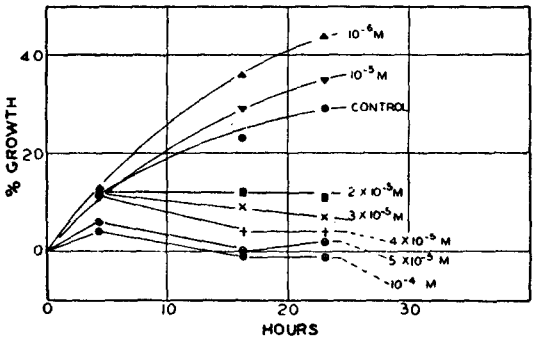


FIG. 2. The effect of various concentrations of iodoacetate on the time course of growth. All solutions contained 1 per cent sucrose and 1 mg. of auxin per liter.

The influence of iodoacetate on the respiration of coleoptile sections was tested over the same range of concentrations. The respiration rates (obtained by Warburg measurements) are plotted in Fig. 3. It is clear

that the effects of iodoacetate on growth and respiration are widely divergent. At a concentration of $5 \times 10^{-5}$ M, while growth is completely inhibited, the rate of respiration is reduced but 9 per cent. Iodoacetate has no marked inhibitory effect on respiration until a concentration of $10^{-4}$ M is reached.

In other words, while iodoacetate at $5 \times 10^{-5}$ M completely blocks the growth processes in the *Avena* coleoptile, it exerts but a small effect on the respiratory processes. It follows that the respiratory requirements of the growth processes cannot represent more than about 10 per cent of the total oxygen consumption, and further that this small fraction of the total

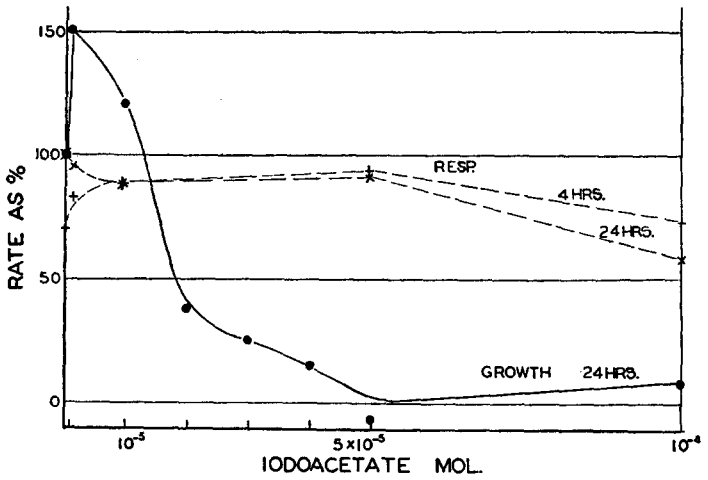


FIG. 3. The effect of iodoacetate on growth and respiration. The growth was measured after 24 hour exposure to solutions containing 1 per cent sucrose, 1 mg. of auxin per liter, and various concentrations of iodoacetate. The respiration rate was determined after 4 hours and after 24 hours.

respiration which is so sensitive to iodoacetate is itself directly concerned with the whole growth process.

### *3. The Nature of the Iodoacetate-Sensitive Process*

The above data have shown that iodoacetate poisons a process (or processes) which, while in complete control of growth, involves but a small fraction of the respiration. The next step was to elucidate the nature of this process.

As a first approach the effect of various substances on the inhibition of growth produced by iodoacetate was studied. The substances tested included those which function as coenzymes or substrates in the enzyme processes which are susceptible of iodoacetate inhibition. These were

added to solutions containing 1 per cent sucrose, 1 mg. of auxin per liter, and $5 \times 10^{-5}$ M iodoacetate. Adenine, nicotinic acid, thiamin, and phosphate had no effect on the iodoacetate inhibition. The positive results obtained are shown in Fig. 4. It is clear that of the variety of substances tested, the four-carbon dicarboxylic acids, and pyruvic acid,[2] are alone able to counteract the inhibition induced by iodoacetate. Comparison of this figure with Fig. 1 shows that these substances even produced their usual acceleration of growth as compared with the sucrose-auxin control; *i.e.*, the entire effect of iodoacetate was nullified.

This result enables us to make at least a tentative identification of the iodoacetate-sensitive process. Since the work of Szent-Györgyi (1935)

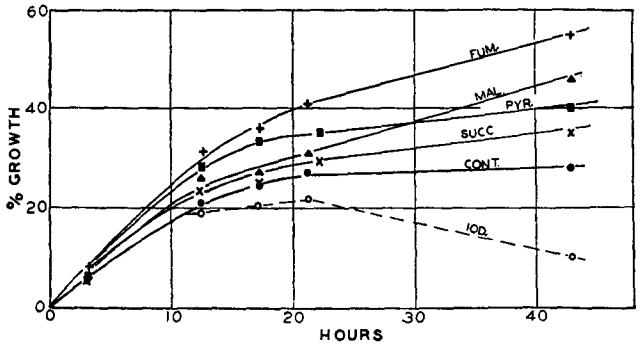


FIG. 4. The effect of four-carbon acids and pyruvate (concentrations: 0.001 M) on the iodoacetate inhibition of growth. All solutions contained 1 per cent sucrose and 1 mg. of auxin per liter. All solutions but the control contained $2 \times 10^{-5}$ M iodoacetate.

it has been known that the four-carbon dicarboxylic acids function as respiratory carriers in most cells. This occurs by way of a series of reversible oxido-reduction reactions:

Succinic ⇆ fumaric ⇆ malic ⇆ oxaloacetic[2]

This sequence serves, in part, to carry the oxidative activity between the cytochrome oxidase and the dehydrogenases. As indicated above, two of the enzymes which mediate these oxido-reduction reactions, malic and succinic dehydrogenases, are inhibited (at least *in vitro*) by iodoacetate. It seems likely therefore that the iodoacetate-sensitive process in the *Avena* coleoptile is the series of four-carbon acid reactions.

[2] The fact that pyruvate behaves in the same way as the four-carbon acids is doubtless due to its participation in the four-carbon acid respiration cycle (Krebs and Eggleston, 1940).

### *4. The Effect of the Four-Carbon Acids on Respiration*

Now, if the above deduction is correct it should be confirmed by an examination of the respiratory activity of the four-carbon acids. It should be possible to show that the coleoptile can oxidize these compounds, and most important of all, that this respiration is in some way controlled by auxin. The experiments which now follow are sufficient, it is believed, to establish these points.

Coleoptile sections which had been soaked overnight in solutions of 1 per cent sucrose plus 1 mg. per liter of auxin, or in sucrose alone, were treated

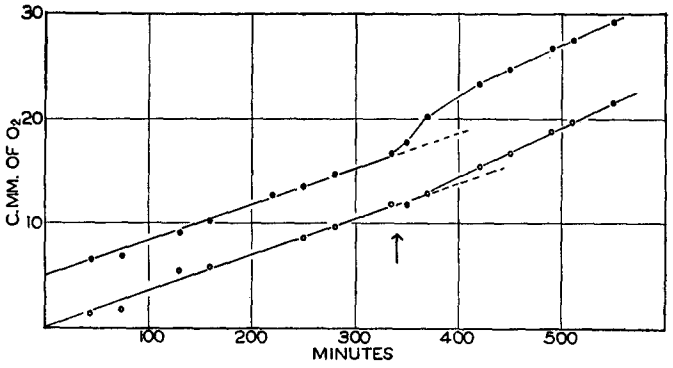


FIG. 5. The influence of auxin on the respiratory effect of malate. The upper curve (closed circles) is for sections which were soaked overnight in 1 per cent sucrose plus 1 mg. of auxin per liter. The lower curve (open circles) is for sections which were soaked overnight in 1 per cent sucrose only. Malate (0.001 M) was added at the arrow mark. The origins are arbitrary.

with 0.001 M malate and the effect on the respiration determined. One set of sections was also soaked in a solution containing sucrose, auxin, and the malate. The respiratory rate of this set was determined on the 2nd day and at the same time the effect of adding malate to the sets soaked in sugar alone and in sugar *plus* auxin was measured. The data presented in Fig. 5 show that when 0.001 M malate is added to the sections, the respiration of those in sucrose alone is increased very slightly, while the sections in sucrose *plus* auxin exhibit a more marked rise in rate.

The presence of malate prevents the fall in respiratory rate which normally occurs during the 1st day after cutting. This decrease in $Q_{O_2}$[3] can be recovered by adding malate on the 2nd day, but the acceleration of

[3] $Q_{O_2}$ as used in this paper, refers to the number of cubic millimeters of oxygen consumed by thirty coleoptile sections during 1 hour.

respiration caused by the malate is much larger when auxin is present than in sucrose alone.

Hence it is apparent that the respiratory activity of malate is augmented by the presence of auxin. This point will be further developed in the sections below.

### 5. *The Respiratory Effect of Auxin*

It is now possible to return to the original problem which was the concern of the earlier workers; namely, what is the effect of auxin on the respiration of the *Avena* coleoptile?

The work of Bonner (1936) and Van Hulssen (1934) showed that growth-accelerating concentrations of auxin have no effect on the respiration of the coleoptiles. All of their determinations were made on large numbers of coleoptiles in water or in a sugar solution. However, the conditions of these experiments did not preclude the possibility that auxin might cause a small and transitory change in $Q_{O_2}$, which could well be masked by the large number of coleoptiles in the respirometer. In fact, the smallness of the fraction of the total respiration which is related to growth lent weight to this suggestion.

Hence these findings were checked by respiration measurements on a single 3 mm. section of a coleoptile in the sensitive microvolumetric respirometer described by Thimann and Commoner (1940). A single section was placed in the respirometer immediately after cutting and floated on 0.1 ml. of distilled water, 1 per cent sucrose, or 1 per cent fructose. After a stable respiration rate had been reached the apparatus was tipped and the section dropped into a solution identical with the original but containing auxin as well. Fig. 6 A which is one of a number obtained in the same way shows that no significant change in the respiration rate can be observed, thus agreeing with the previous work on larger masses of tissue.[4]

However, the importance of the four-carbon acids, made clear above, suggested that the respiratory effect of auxin (if any) might be dependent on the presence of these substances. Consequently, the above experiment was repeated by adding auxin to a section which had been previously soaked for several hours in a solution containing 1 per cent sucrose and 0.001 M K malate. Fig. 6 B shows that an increase in $Q_{O_2}$ of about 14 per cent results from the addition of auxin in a concentration of 1 mg. per liter.

[4] Du Buy and Olson (1940), using another method, also found little change in respiration when 1 mg. per liter indole-acetic acid and fructose was added to freshly cut coleoptiles. Their published curve appears, however, to show a slight increase (*cf.* also Fig. 7).

The rise in respiratory rate occurs almost immediately and is noticeable about 15 minutes after the addition takes place.

Since this effect appeared to be stable over a period of several hours, it was possible to investigate it more fully using larger masses of tissue and Warburg respirometers.

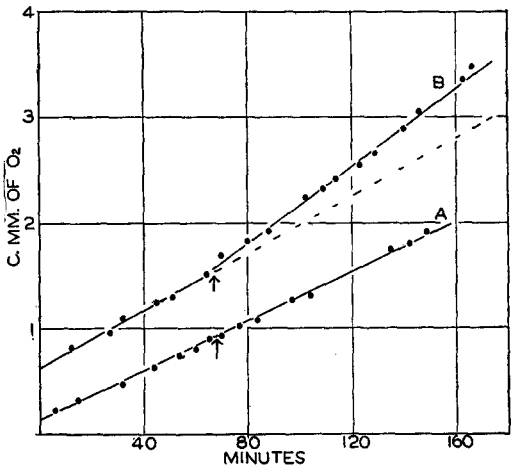


FIG. 6. The effect of auxin on the respiration of freshly cut apical sections of the *Avena* coleoptile. Curve A is for a single section placed in 1 per cent sucrose immediately after cutting, curve B for a single section in 1 per cent sucrose plus 0.001 M malate. Auxin (1 mg. per liter) was added at the point marked by the arrow. It should be noted that these $Q_{O_2}$'s are high as compared with later measurements made with larger masses of tissue. This is due to the fact that the apical sections have the highest normal $Q_{O_2}$ and that later measurements were made on sections obtained from 9 mm. (subapical) of the coleoptile. The latter therefore have a lower average respiration. The present measurements were made immediately after cutting so that the fall in $Q_{O_2}$ which occurs on standing was avoided. The origins of the curves are arbitrary.

Thirty 3 mm. sections of *Avena* coleoptiles were introduced into the main compartment of the Warburg vessel together with 1.5 ml. of one of the following media: distilled water, 1 per cent sucrose, 1 per cent sucrose plus 0.001 M malate, 1 per cent sucrose plus 0.001 M fumarate. Into the side-arms were introduced 0.5 ml. portions of a solution of auxin in the appropriate medium. The auxin concentration was 4 mg. per liter, thus making 1 mg. per liter when added to the main part of the vessel. In the case of the water experiments, two side-arms were used, one containing a solution of malate (to make 0.001 M when added) and the other a solution of auxin (to make 1 mg. per liter when added).

The sections were soaked in the appropriate medium overnight (*i.e.* the medium to be placed in the main compartment of the vessel). 12 hours after sectioning the respiration rates were measured, and after $4\frac{1}{2}$ hours the side-arm solutions were tipped in. The respiration rate was followed for a number of hours after tipping. The results, presented in Fig. 7, offer clear-cut data on the effect of auxin on the respiration of coleoptile sections:

1. The addition of auxin to sections respiring in water caused no significant change in the rate of respiration (curve A).

2. Sections soaked in sucrose (B, C, D) show an increase in $Q_{O_2}$ of from 8 to 13 per cent (average 10 per cent) on addition of auxin. This increase does not occur until about 1 hour after the addition takes place. In other experiments where the time of soaking in sucrose was less, this increase did not occur at all.

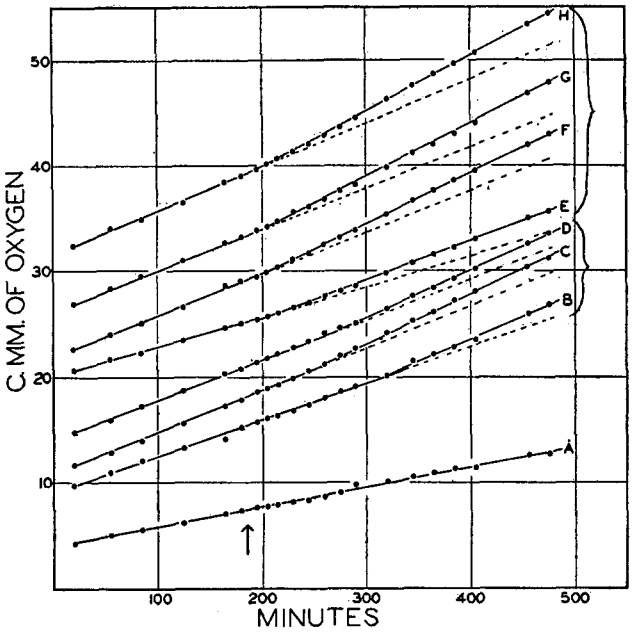


FIG. 7. The effect of auxin on the respiration of sets of thirty coleoptile sections (from ten coleoptiles) under various conditions. A, in water; B, C, D, in 1 per cent sucrose; E, F, G, in 1 per cent sucrose plus 0.001 M malate; H in 1 per cent sucrose plus 0.001 M fumarate. Auxin (1 mg. per liter) was added at the arrow mark. The origin of each curve is arbitrary.

3. On the other hand, the addition of auxin to sections kept in a mixture of sucrose and malate (E, F, G), or in sucrose plus fumarate (H) produced an increase in respiration rate of from 15 to 28 per cent (average 22 per cent).

It was also found that the addition of malate (0.001 M) to water-soaked plants caused no significant change in the rate of respiration.

Now it was shown that the respiratory activity of malate depends on the presence of auxin, and the data of Fig. 7 show that auxin can itself stimulate respiration. This stimulation occurs to a small extent if the

sections have been exposed to sucrose for a long time, but is greatly magnified by the presence of malate or fumarate. There is thus a mutual relationship between the effect of auxin and the four-carbon acids on respiration. If we bear in mind as well the growth experiments of Figs. 1 and 4, it is clear that the four-carbon acid respiration system must be one of the links in the chain of growth processes.

If this is true, the effect of auxin in stimulating growth should parallel closely its effectiveness as an activator of the four-carbon acid respiration.

### *6. The Identity of the Auxin Effect on Growth and on the Four-Carbon Acid Respiration*

The acceleration of growth produced by auxin has long been known to be closely dependent upon the concentration of the hormone. Consequently, the problem of the possible identity of the two auxin effects was studied by determining the variation of their intensities with different concentrations of auxin.

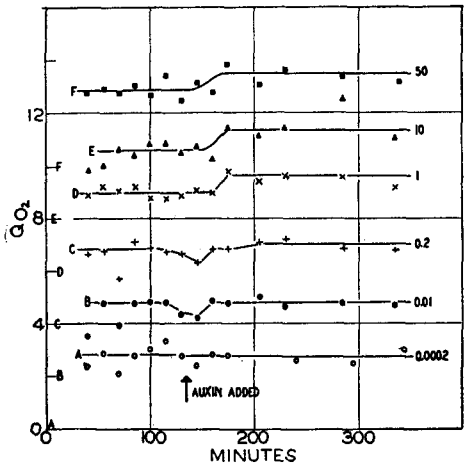


FIG. 8. The effect of various concentrations of auxin on the $Q_{O_2}$ of thirty coleoptile sections. The concentration of auxin in milligrams per liter is indicated on the right of each curve. The zero point for each curve is marked by the position of the letter corresponding to it on the ordinate. For example, the initial $Q_{O_2}$ of curve C is 2.8. All solutions contained 1 per cent sucrose plus 0.001 M malate.

Sets of thirty sections were soaked overnight in a solution of 1 per cent sucrose plus 0.001 M malate and placed in Warburg vessels with 1.5 ml. of the same solution. The side-arms were filled with 0.5 ml. of the mixture made up to contain various auxin concentrations. The effect of adding varying amounts of auxin is shown in Fig. 8 (not all of the concentrations used are shown here). On the 3rd day the sections were removed from the vessels and their lengths measured. The percentage increase in length is plotted in Fig. 9 along with the respiration data of Fig. 8 reduced to percentages.

It is clear from this figure that there is a qualitative identity between the effect of auxin on growth and on the four-carbon acid respiration. The curves are decidedly parallel, both showing optima in the range of 1 to 10 mg. of auxin per liter.

### 7. *Characteristics of the Respiratory Processes Involved in Growth*

In order to study further the properties of the four-carbon acid respiration in the coleoptile it was necessary to deplete the tissues of their reserves of respiratory substrates. This procedure would then reduce the activity of the total respiration, and enable the normally small fraction of four-carbon acid respiration to be measured with greater accuracy. It was shown in Fig. 7 that on soaking the sections in water (or in sucrose solution) the respiratory rate falls off, and that this falling off is prevented by the presence of sucrose and malate together. It may be deduced from

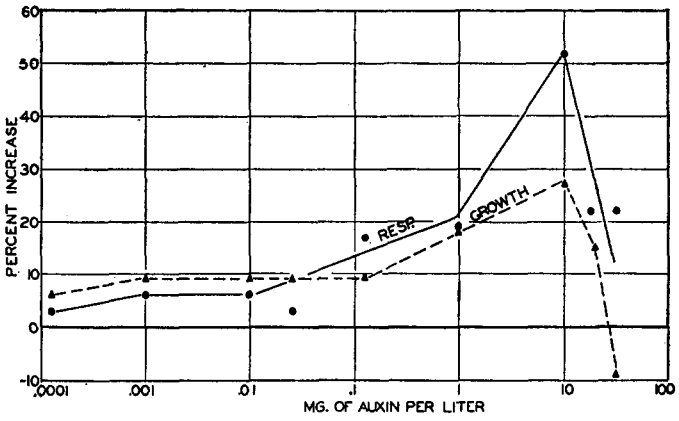


FIG. 9. The effect of various concentrations of auxin on the growth and respiration of coleoptile sections. The data were obtained on the same sets of thirty sections each. The increases noted are relative to the $Q_{O_2}$ and coleoptile length previous to the addition of auxin. All solutions contained 1 per cent sucrose + 0.001 M malate.

this that during soaking in water the reserves of malate (and the other four-carbon acids) are depleted. Hence, if such substances were then added to these sections, the measurement of the resultant respiratory rate could provide an accurate index of the activity of this normally small fraction of the total respiration.

Such experiments were carried out by soaking the sections in distilled water for a period of about 18 hours. At the end of this time the $Q_{O_2}$ (per thirty sections) had fallen to 1.2, less than one-half of the original value. The effect of the four-carbon acids under various conditions was then determined by measuring the $Q_{O_2}$ after the various substances had been added to the starved coleoptile sections. All the solutions used contained 1 per cent sucrose.

The results obtained from this experiment are shown in Fig. 10. Fig.

10*a* shows the effect of various concentrations of malate (in the presence and absence of auxin) on the respiration and growth of the coleoptile sections. If auxin is present, the respiration is markedly dependent upon the concentration of malate, rising to an optimum at 0.001 M and falling off to a low and constant value at higher concentrations. However, if there

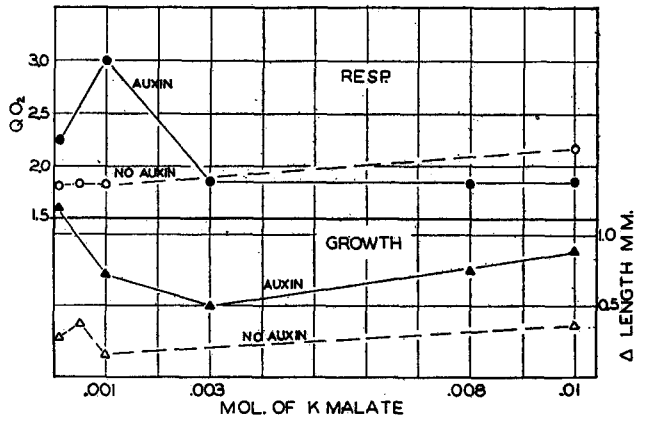


FIG. 10*a*

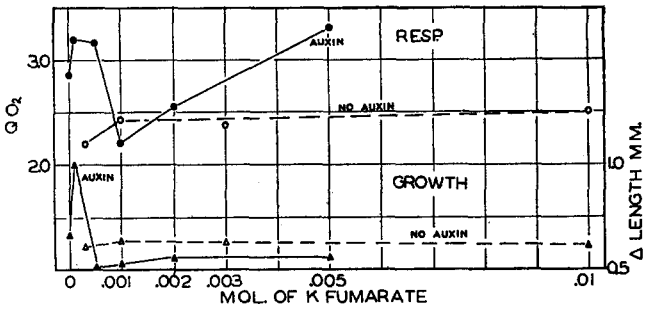


FIG. 10*b*

FIG. 10. The effects of various concentrations of malate (*a*) and fumarate (*b*) on growth and respiration of coleoptile sections. The growth and respiration measurements were made on the same sets of sections in each case. The auxin concentration used was 10 mg. per liter. All solutions used contained 1 per cent sucrose.

is no auxin present, the $Q_{O_2}$ is almost entirely independent of the malate concentration, the rate remaining at the low level characteristic of the supra-optimal concentration of malate in the presence of auxin. Similarly, the length increments,[5] which roughly parallel the $Q_{O_2}$, are dependent on malate only in the presence of auxin.

[5] It should be pointed out that such starved sections exhibit a smaller response (in growth) to the addition of auxin as compared with freshly cut sections.

The effect of various concentrations of fumarate (in 1 per cent sucrose) on growth and respiration is of a similar nature (Fig. 10*b*). In this case too, the stimulations of growth and respiration parallel each other, at least in the lower concentrations, and occur only if auxin is present.

Thus, when auxin is present, malate and fumarate appear to exert a catalytic effect on the oxidation of the sucrose in the medium. This catalytic effect is indicated by the shapes of the curves, which rise to an optimum $Q_{O_2}$ and then fall off sharply. This suggests that small amounts of these acids can bring about an increase in respiration not by being themselves oxidized, but rather by stimulating the oxidation of some other substrate—such as sucrose. Such an interpretation is of course in keeping with the well established rôle of the four-carbon acids as *carriers* of oxidative processes in the cell.

In the case of fumarate the optimum concentration is somewhat lower than that of malate, and a secondary rise in $Q_{O_2}$ occurs in concentrations greater than 0.001 M. This latter phenomenon seems to indicate that in addition to its catalytic effect on the oxidation of other substrates, fumarate may itself be irreversibly oxidized if present in high concentrations. It is interesting to note, however, that the secondary rise in rate although it is dependent on auxin, does not occur in the effect on growth. Less complete experiments with succinate gave results similar to those obtained with fumarate.[6]

It appears therefore, that in the *Avena* coleoptile the functioning of malate and fumarate as respiratory carriers is dependent on the presence of auxin. Furthermore the catalytic stimulation of respiration by these substances is paralleled by their acceleration of growth. It seems clear that the respiratory activity of the four-carbon acid system is in some manner catalyzed by auxin, and that this activity is one of the requisites for the stimulation of growth by auxin.

## IV

## DISCUSSION AND CONCLUSIONS

The data here presented provide the basis for a new understanding of the relationship between growth and respiration in the *Avena* coleoptile.

Perhaps the most important conclusion to be drawn is that the effectiveness of auxin as a growth hormone is closely related to its effect on certain respiratory processes; *i.e.*, that auxin itself provides the link between growth and respiration.

[6] As a check on these experiments, a similar run with various concentrations of acetate was carried out. There was no significant effect on the growth or respiration.

When it was first discovered that the growth of plants depended on their respiratory activity, the latter was looked upon as a "primary essential" for growth (Pfeffer, 1900); that is, growth depended on the energy derived from respiration, and so it was logically necessary that respiration be required for growth. This concept of generalized dependence received support, in more recent times, from the finding of Bonner (1936) that cyanide inhibition reduced growth and respiration proportionally.

The present paper indicates that this relationship, rather than being formal, is mediated by certain very specific processes. It has been shown that growth may be completely inhibited by the proper concentration of iodoacetate and the respiratory rate reduced by but 10 per cent. Thus there must exist some process which, while responsible for but a small part of the normal respiration, is wholly in control of growth and of the effect of auxin on growth. This finding agrees with the conclusion of Sweeney and Thimann (1938) that auxin accelerates a respiratory process representing only a small part of the total $Q_{O_2}$ but controlling protoplasmic streaming and growth.

It has also been shown that this link is represented by the four-carbon acid respiratory system. This system catalyzes *part* of the total oxidation of respiratory substrates such as sucrose (probably in the form of glucose) but is apparently a direct link in the chain of reactions which is responsible for *all* of the growth.

The four-carbon acid respiration system represents a small but variable fraction of the total $Q_{O_2}$. In the freshly cut section it accounts for but 10 per cent of the total; and thus, since it alone responds to the addition of auxin, no detectable increase in $Q_{O_2}$ occurs when the hormone is added under these conditions. However, if malate or fumarate is soaked into the coleoptile section, the respiratory *capacity* of this system becomes enlarged, so that when it is activated by addition of auxin a noticeable increase in respiratory rate ensues.

The effect of various concentrations of malate and fumarate indicates that these substances catalyze the oxidation of other respiratory substrates (such as sucrose), although in the case of fumarate, and perhaps succinate, there is evidence to indicate that they may themselves be irreversibly oxidized when present in sufficiently high concentrations. It was not the purpose of the present paper, however, to attempt a detailed analysis of the properties of this respiratory system, but rather to discover the qualitative linkage between growth and the respiratory processes related to it.

The data obtained indicate that the dependence of growth on oxygen consumption is due to the participation of a respiratory process, the four-

carbon acid system, in the chain of growth processes. Further, it is clear that the influence of auxin on growth is related to its effect on this respiratory system.

## V

## SUMMARY

1. The growth of *Avena* coleoptile sections in sucrose and auxin solutions is inhibited by various substances which are known to act as dehydrogenase inhibitors.

2. Iodoacetate, which is particularly active in this connection, inhibits all growth at a concentration of $5 \times 10^{-5}$ M, but produces only a slight inhibition of oxygen uptake.

3. The growth inhibition by iodoacetate is completely removed by malate and fumarate, and to a lesser extent by succinate and pyruvate.

4. These acids themselves increase the effect of auxin on growth and also increase the respiration of the coleoptile sections, but only if auxin is present.

5. When sections have been soaked in malate or fumarate, the addition of auxin considerably increases the total respiration. Further, the concentration range over which this increase takes place parallels that active in promoting growth.

6. The four-carbon acids provide a respiratory system which is part of the chain of growth processes, and which is in some way catalyzed by auxin. It represents a small but variable fraction of the total respiration.

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