THE GROWTH AND RESPIRATION OF THE AVENA COLEOPTILE

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I

INTRODUCTION

In a previous paper (1) a relation was shown to exist between the respiration of the plant cell and its elongation under the influence of the plant growth hormone. A more extensive investigation of this relation was therefore undertaken with the hope that elongation would exhibit a close correlation with some relatively accessible property of the respiration, for example with the magnitude or the respiratory quotient of the latter. It may be said at once, however, that this was not the case, and that the work reported in the present paper, while revealing several points of interest and defining more clearly the dependence of elongation upon respiration, has not resulted in any explanation of the way in which respiration is essential to growth.

II

Materials and Methods

Avena coleoptiles from seedlings of the pure line "*Sieges Hafer*" were used throughout. They were grown under the usual controlled conditions (2). The measurements of respiration were made by the method of Warburg with the apparatus of Dr. Robert Emerson to whom the author is greatly indebted. Conical vessels possessing a central alkali well and side wells for reagents to be added during the course of an experiment were used. Twenty coleoptile sections (1) suspended in the desired solution were placed in each vessel. The measurements of elongation were carried out in the manner described in the previous paper (1).

III

Steps of the Growth Process Dependent upon Respiration

It was earlier shown that coleoptile sections do not elongate in growth substance solution when placed in an atmosphere of oxygen-free nitrogen. When such sections were subsequently placed in growth substance solution in air they elongated rapidly, showing that they had not been harmed. If they were, however, placed in water without growth substance they did not elongate, showing that they had not taken up and stored growth substance; that is, that the *transport* of the hormone into the sections does not take place in the absence of aerobic metabolism. In order to show that the action of growth

TABLE I

Inhibition of the Action of Growth Substance Already in the Plant

Agar block (2 hrs.)	Subsequent solution	Subsequent elongation
		per cent in 8 hrs.
Water agar	Distilled water	3.4
Water agar	10 u./cc.	27.6
1200 plant units	Distilled water	12.8
1200 plant units	KCN, 3×10^{-3}N	2.1
1200 plant units	KCN, 1×10^{-3}N	3.7
1200 plant units	Phenylurethane, 0.05 per cent	4.9

substance already *in* in the section is also inhibited under these conditions, the following type of experiment was performed. Two lots of coleoptile sections were prepared. Upon the apical ends of one lot were placed agar blocks containing no growth substance. Upon the apical ends of the other set were placed agar blocks containing 1200 plant units of the hormone. The sections were left at 25°C in a saturated atmosphere for 2 hours. At the end of this time some of those which had had no growth substance were placed in water, the remainder in a solution containing 10 units of growth substance per cubic centimeter. The sections which had had 1200 plant unit blocks were distributed among the following solutions, KCN, 3×10^{-3}N; KCN, 1×10^{-3} N; phenylurethane, 0.05 per cent; water. Table I gives the results of a typical experiment. There is no question but that growth

substance passed into the sections which had had 1200 plant unit blocks, since these sections elongated more in water than did those which had had plain agar and were also placed in water. This elongation brought about by growth substance already in the plant is, however, clearly inhibited by both KCN and phenylurethane. We must conclude, therefore, that both growth substance *transport* and growth substance *action* are dependent upon aerobic metabolism.

IV

Effect of Growth Substance upon the Magnitude of Respiration

In the earlier paper (1) it was shown that crude growth substance preparations cause an increase in the rate of respiration of coleoptile

TABLE II

*Effect of Crystalline Auxine B on Coleoptile Respiration**

Solution	Q_{O_2} before addition	Q_{O_2} after addition
0.0 units, *per cc.*	0.70	0.60
10 units, *per cc.*	0.70	0.60

* In this and in all subsequent tables, Q_{O_2} denotes mm.^{3} of oxygen consumed per section per hour, measured at standard conditions of temperature and pressure.

sections. It was suggested that although this increase might prove to be due to the hormone itself, it might on the other hand be due to associated impurities. The latter supposition can now be shown to be the correct one since, as has also been found by Kögl (3) the respiratory stimulant is absent from crystalline auxine and can be removed from crude hetero-auxine (4) preparations by further purification. Table II shows that crystalline auxine B (kindly supplied by Professor F. Kögl, Utrecht) does not affect coleoptile respiration. Table III shows that while a crude hetero-auxine preparation (from cultures of *Rhizopus suinus* (4)) having an activity of 500,000 plant units per milligram causes a considerable increase in respiration, a purification of this extract to 1,500,000 plant units per milligram removes most of the stimulating power.

The respiratory effect of the more impure hormone preparation is

probably due to a specific stimulating substance rather than to a nutritive action of the associated impurities for the following reasons; (*a*) it has been found that fructose, which should also have a nutritive action, has but little effect upon coleoptile respiration under the conditions used and (*b*) it has been shown (1) that the stimulating substance is destroyed by oxidation with H_2O_2.

That growth substance does not influence the magnitude of the respiration may be demonstrated in another manner. As has been described elsewhere (2) the activity of growth substance in promoting cell elongation depends upon the undissociated growth substance present. However, the pH of the cell contents is such that only 4 to 5 per cent of the hormone present in the plant is in the active form. If the cell acidity is increased by placing the coleoptile section in an acid

TABLE III

Effect of Crude Hetero-Auxine Preparations upon Respiration

Solution of	Q_{O_2} before addition	Q_{O_2} after addition	
		1 hour	2 hours
10 units *per cc.* (2500 units, or 500,000 plant units, *per mg.*)	1.56	1.92	1.95
10 units *per cc.* (7500 units, or 1,500,000 plant units, *per mg.*)	1.53	1.63	1.43

buffer solution, more of the cell's own growth substance becomes active and its rate of elongation is increased even without the addition of more growth substance. If, on the other hand, the section is placed in a neutral or basic buffer solution, the acidity of the cell contents becomes less than normal, growth substance is dissociated, and the rate of elongation of the section is decreased. A comparison of the respiratory rates in buffer solutions of pH 4.1 and pH 7.2 was therefore made. Since the buffer of pH 7 retains a portion of the CO_2 given off by the respiring tissue it was necessary to add an excess of acid at the end of each experiment, in order to drive off all of the accumulated CO_2 into the gas space of the vessel, where it could be absorbed by the KOH of the alkali well. Table IV shows that there is no difference in the respiratory rates at the two pH values although the rates of

elongation are greatly different. It is clear, then, that growth substance has no effect upon the magnitude of coleoptile respiration, a conclusion also reached by Kögl (3).

Since changes in the growth rate of the coleoptile induced by growth substance are not paralleled by changes in the intensity of respiration

TABLE IV

Effect of pH upon the Elongation and the Respiration of Coleoptile Sections

	pH 4.1	pH 7.2
Elongation in 2 hrs., *per cent*	9.4	2.7
Q_{O_2}	1.53 ± 0.05	1.49 ± 0.05

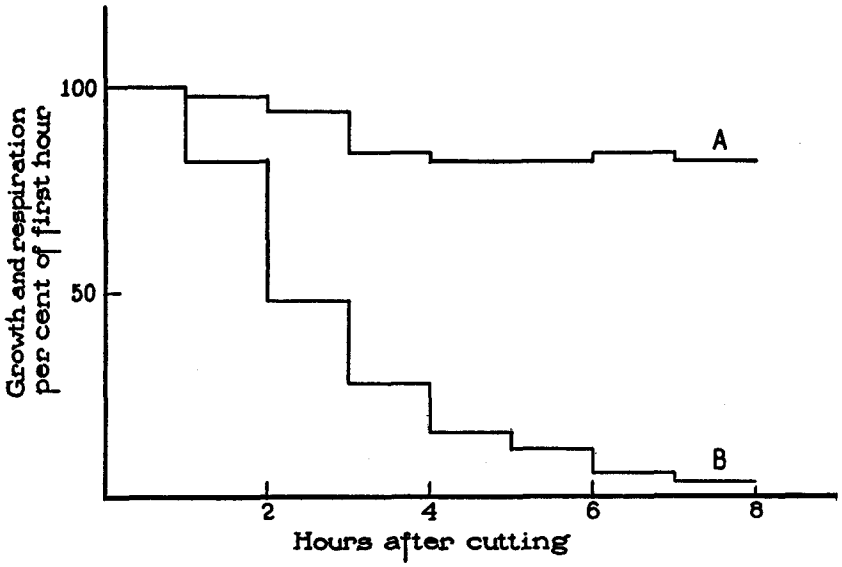

FIG. 1. Changes in the growth rate and in the respiration rate of coleoptile sections with time. Curve A, respiration rate; Curve B, growth rate.

it might be expected that changes in the growth rate induced by other causes should also be without effect. Fig. 1 compares graphically the changes with time of (*a*) the growth rate, and (*b*) the rate of respiration, of sections immersed in growth substance solution. The growth rate even in the presence of excess growth substance decreases steadily

until after 8 hours it practically reaches 0. At this time then, substances or conditions other than the growth hormone are limiting elongation. The rate of respiration, on the other hand, sinks only about 20 per cent in this time. Similarly, the distribution of growth rate and respiratory rate over the length of the coleoptile do not parallel one another markedly, as is shown in Fig. 2. Even when the

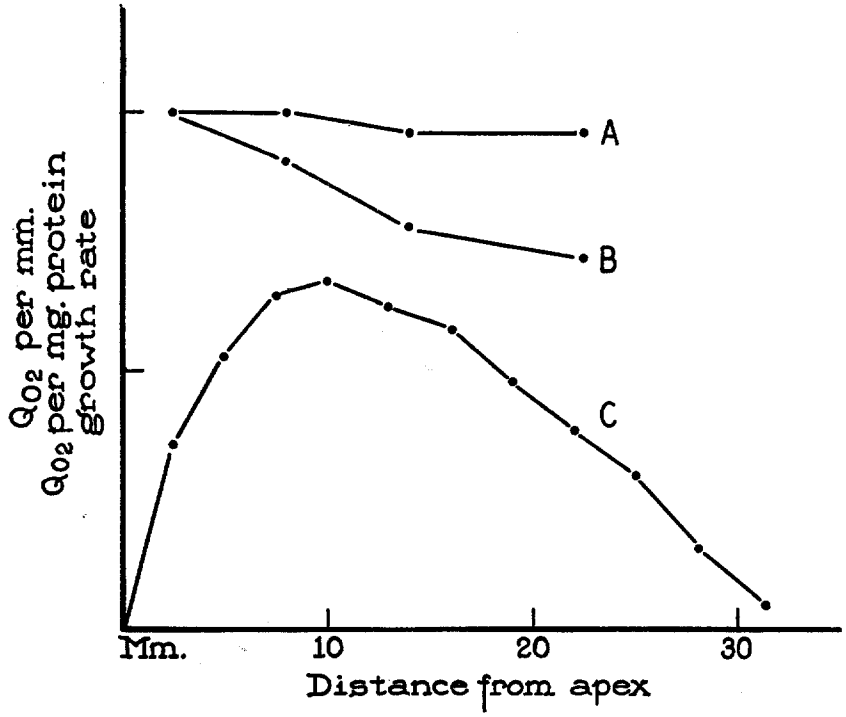

FIG. 2. Distribution of growth rate and of respiration rate in the coleoptile. Curve A, rate of respiration per milligram; Curve B, rate of respiration per milligram protein; Curve C, growth rate (after Went (9)).

respiration per milligram protein is used as the basis of comparison, the respiration of the slowly growing base of the coleoptile is 80 per cent of that in the zone of maximum growth rate. Although there is in these two cases no strict parallelism between elongation and respiration, still it is to be noted that a slight decrease in respiratory rate is accompanied by a large decrease in growth rate.

V

Does Growth Substance Change the Nature of the Respiration?

A search for qualitative differences in the nature of the respiration in the presence and in the absence of growth substance was next made. The respiratory quotient of sections under different conditions was determined by the method of Warburg. This method consists in the measurement of the gas which may be driven out of the tissue by acid at the beginning of the experiment ("preformed CO_2"), the net gas exchange, the gas driven off by acid at the end of the experiment, and the oxygen consumption during the experiment. The determination

TABLE V

Effect of Growth Substance upon the Respiratory Quotient

Solution	Total gas exchange mm.³	O_2 uptake mm.³	R.Q.
pH 4.1	+51.3	175.0	1.29
pH 4.1 + growth substance	+51.2	175.0	1.29
pH 7.2	−41.1	243.0	0.83
pH 7.2 + growth substance	−43.5	243.0	0.82
1 per cent fructose	−9.1	228.0	0.96
1 per cent fructose + growth substance	−8.1	228.0	0.96

of the respiratory quotients revealed a peculiar fact which has, however,—so far as can be seen at present,—no relation to the action of growth substance, namely, that the respiratory quotient is markedly higher for sections suspended in an acidic buffer than for sections suspended in a neutral buffer. In buffer of pH 4.1 the quotients are very constantly greater than one, in buffer of pH 7.2 they are more irregular but are markedly below one. It must be emphasized that the fact that during the main body of the experiment most of the CO_2 is driven off by buffer of pH 4.1 and a portion of it retained by buffer of pH 7.2 does not affect directly the determination of the respiratory quotient since in both cases all of the CO_2 is ultimately driven off with excess acid. Moreover, this difference in respiratory quotient between acid and neutral solutions has no direct relation to growth, since, as is shown in Table V, in unbuffered fructose solution the quotient is the same irrespective of the elongation, and is practically

one, as would be expected. The sample experiment of Table V shows that in buffer of pH 4.1 the R. Q. is greater than one, in buffer of pH 7.2 less than one, and in unbuffered solution approximately one, and that in all three of these cases growth substance is without effect. In spite of the unexplained difference between acidic and neutral solutions it seems safe to believe that growth substance itself has no influence upon the respiratory quotient.

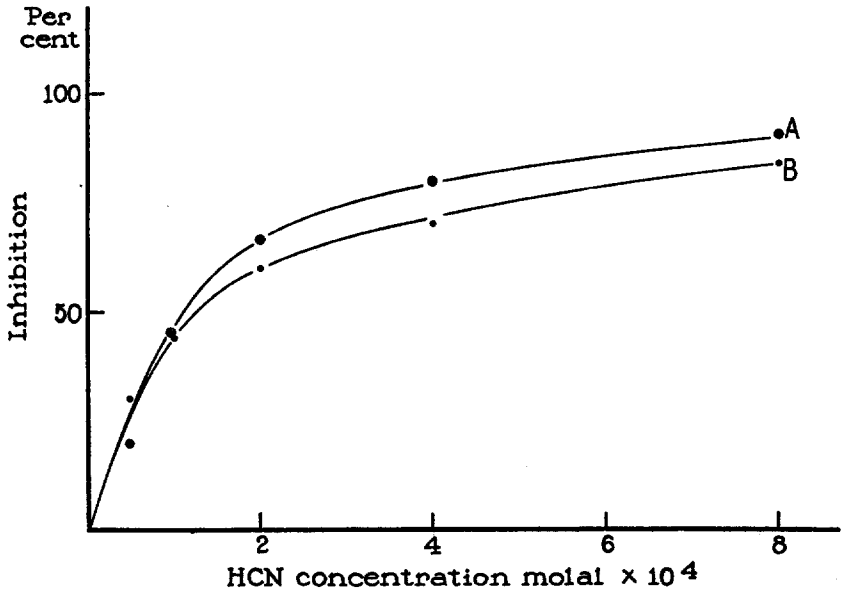

FIG. 3. Inhibition of growth and of respiration by HCN. Curve A, inhibition of respiration; Curve B, inhibition of growth.

Strugger (5) has suggested that fermentation favors elongation. The high quotient at pH 4 might indeed be interpreted as a kind of fermentation. However, this high quotient is not essential to growth. Moreover, if fermentation were conducive to cell elongation one might expect that anaerobiosis should stimulate growth, or that HCN in suitable amounts should, by inhibition of the "Pasteur reaction," increase elongation. An increase of growth rate by either anaerobic conditions or HCN has never been observed in the course of these experiments.

VI

Effect of HCN on Growth and on Respiration

Up to the present point the independence, both quantitative and qualitative, of cell elongation and respiration has been stressed. In Part III and in the earlier paper (1) it has, however, been pointed out that this independence is by no means complete, since, for example, HCN concentrations of about the same order are needed to inhibit the two processes. A more quantitative study of this relation has now been made and the results are presented in Fig. 3, in which per cent inhibition is plotted against HCN concentration. The two curves parallel one another very closely. A similar parallel inhibition of growth and of respiration was demonstrated earlier for phenylurethane (1).

VII

DISCUSSION

The experimental results which have been presented show that: (*a*) during the growth of the cell, either in the normally growing coleoptile or in the excised coleoptile section, the rate of elongation decreases much more rapidly than does the rate of respiration; (*b*) HCN and phenylurethane decrease elongation and respiration to the same extent. Low temperatures also slow down both processes, although respiration is somewhat more affected than is elongation (6). Neutral buffers, however, decrease elongation without exerting any effect upon respiration.

From (*a*) and (*b*) it is necessary to conclude that decreases in respiration decrease elongation but not the reverse; *i.e.*, no considerable portion of the respiration is the *result* of elongation processes. This dependence of growth upon a large respiration may be viewed in two ways; (1) the entire respiration is a "formal prerequisite" of growth (Pfeffer (7)); (2) one or more of the processes of growth is itself a respiratory process but one of small oxygen uptake. Calculations of the amount of mechanical work done during cell elongation have shown that only a small portion of the energy liberated during respiration can be used in this way, and many workers have shown that when the respiratory quotient is near one, the energy of respiration all

appears ultimately as heat (Algera (8)). (1) can give therefore no clear idea of why a reduction of respiration by HCN or phenylurethane should decrease elongation to even approximately the same extent. This brings up the question as to the way in which respiration might be a formal prerequisite of growth. It is clear that it is necessary only if it performs functions which are necessary for growth. The principal function which it performs is the production of heat, which it is difficult to visualize as useful for elongation. It seems more likely therefore that only a portion of the respiration is needed in the actual growth processes, and (1) would become identical with (2).

Experimentally also the distinction between (1) and (2) is difficult if not impossible. (1) Growth is decreased when respiration is decreased because the whole respiration is essential. (2) Growth is decreased when respiration is decreased because the two portions of the respiration have similar properties. Upon (1) elongation can cease and respiration continue because the latter is a "prerequisite" of the former. Upon (2) elongation can cease and respiration continue because of the inhibition of other portions of the growth process.

Concept (1) therefore cannot be either theoretically or experimentally strictly differentiated from concept (2). It would seem desirable then to avoid the ambiguity of meaning in statement (1), and to express the necessity of respiration for growth in the statement that a process of respiratory nature is one (or more) of the component processes of elongation.

VIII

SUMMARY

1. *Transport* of the plant growth hormone into the *Avena* coleoptile as well as the *action* of the hormone on cell elongation in the coleoptile are shown to depend upon aerobic metabolism.

2. Crystalline auxine, in contrast with impure preparations, affects neither the magnitude nor the respiratory quotient of coleoptile respiration.

3. Increasing age of the coleoptile cell decreases its rate of elongation much more than its rate of respiration. HCN or phenylurethane on the other hand decrease the two processes to the same extent, in spite

of the fact that only a small portion of the energy liberated by respiration can be used in the mechanical process of growth.

4. From 2 and 3 it is concluded that processes of a respiratory nature but of relatively small magnitude form one or more integral steps in the chain of reactions by which the plant growth hormone brings about cell elongation.

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