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THE EFFECT OF INDOLE ACETIC ACID AND OTHER GROWTH PROMOTING SUBSTANCES ON THE ENDOGENOUS RESPIRATION OF THE AVENA COLEOPTILE

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CONTENTS

GENERAL INTRODUCTION

§ 1. AEROBIC RESPIRATION AS A "FORMAL PREREQUISITE" FOR GROWTH

The first investigations, indicating that growth by cell elongation is connected with the aerobic respiration, date back to the last century.

WIESNER (1878) reported that "heliotropische Erscheinungen" (phototropic curvatures) in seedlings of *Phaseolus multiflorus*, *Vicia faba* and other plants only occurred when there was free oxygen in the environment.

WORTMANN (1880) noticed the same with the roots of seedlings of these plants: without free oxygen growth and geotropic response failed to occur.

Analogous results were obtained by CORRENS (1892) with various seedlings and by VAN AMEYDEN (1917) with *Avena* coleoptiles: in a nitrogen atmosphere neither phototropic nor geotropic curvatures took place.

Aerobic respiration was understood in those days as a "primary essential" (PFEFFER, 1900) for growth in the way that respiration was merely the source of energy for the growth processes, which appears, for instance, from WORTMANN's statement (l.c. p. 520) of the problem. This author asked himself: "weshalb die intramoleculare Athmung allein für die Pflanzen keine Kraftquelle ist, weshalb nur durch das Eingreifen des atmosphärischen Sauerstoffes die Kräfte frei werden, welche das Wachstum bewirken" (why in plants the intramolecular respiration does not suffice as a source of energy, why only by the action of atmospheric oxygen the energy is set free, which causes growth).

A new attack on the problem of the relation between growth and respiration was made after the discovery of the growth substances. The early statement of WENT (1928) that growth substances are in-

dispensable for growth has been more and more widely accepted and many investigations were started to detect the locus as well as the mode of action.

Originally, auxin action was localised in the cell wall (HEYN, 1931; RUGE, 1937, 1942). Then the protoplasmic membrane was considered as the probable locus of action (KONINGSBERGER, 1942, 1947; VELDSTRA, 1944). Finally, the auxins were thought to be active inside the protoplasm. Recent literature does not leave much doubt, that growth substances cause physico-chemical and (or) chemical changes in nearly all parts of the cell. This does not include, however, that all these changes are supposed to be the result of a direct action on the spot.

In theories on growth substance action one often meets with the view that they do not act on different cell components separately but that their action is confined to control in some way a master system. The diverse phenomena resulting from the supply of a growth substance would have to be considered as secondary reactions which were started by, what THIMANN (1935) called, "the master reaction". A distinction was therefore made between the primary effect of a growth substance, which was thought to act upon a hypothetical master system, and diverse secondary reactions.

COMMONER and THIMANN (1941) considered the primary action to be exercised on the respiration. Growth would be closely linked to that part of the respiratory process which is called the SZENT GYORGYI — KREBS- or C_4-dicarboxylic cycle. The authors showed that the growth of *Avena* coleoptile sections in sucrose and auxin solutions was inhibited by substances which are known to inhibit dehydrogenases. Particularly, iodoacetate appeared to be very active in this respect as, with a concentration of 5×10^{-5} M growth was stopped entirely, whereas the respiration was only reduced to about 10 %. The growth inhibition could be neutralized by addition of components of the above-called cycle (malate, fumarate and succinate) and also by pyruvate. Besides, addition of these acids increased the growth promoting influence of indole acetic acid (I.A.A.). Further, the respiration of sections, soaked in malate or in fumarate, was increased from 15–28 percent by I.A.A. It was concluded that the "four-carbon acids provide a respiratory system which is part of the chain of growth processes, and which is in some way catalized by auxins. It represents a small but variable fraction of the total respiration" (l.c. p. 295).

In this theory the respiration is *placed into* the chain of processes which result in growth. Instead of being only a "formal prerequisite", an energy providing process, it is held that substances which promote the growth act *via* the respiration.

The ideas of COMMONER and THIMANN have been further developed by THIMANN and BONNER (1948, 1949, 1950) in a series of papers on the growth and inhibition of isolated plant parts (*Avena* coleoptile sections and *Pisum* internodes). Experiments with more specific sulfhydril inhibitors confirmed earlier investigations and led to the final

conclusion that growth is controlled by an enzyme (or co-enzyme) which contains a sulfhydril group, and that the organic acids of the KREBS-cycle represent the central link between the diverse metabolic processes which are involved in growth (see also THIMANN, BONNER and CHRISTIANSEN, 1951).

In a recent communication on the mechanism of auxin action THIMANN (in SKOOG, 1951) considered auxins as ideal protectors of the organic acid metabolism against natural inhibitors. According to his opinion auxin combines with the enzyme to be protected. The adsorption power to this enzyme must necessarily be stronger than that of the hypothetical inhibitor.

In spite of the criticism on the above-mentioned theory (see BERGER and AVERY, 1943, who showed that their results were open to other interpretations), the significance of the organic acid metabolism for growth has been recognized even by those authors who localized the auxin action in the phosphate metabolism (see later).

§ 2. THE INFLUENCE OF GROWTH SUBSTANCES ON THE OXYGEN UPTAKE

A special aspect of the problem of the relation between growth, growth substances and the aerobic respiration, which has been studied in many investigations, including the present one, is that of the ability of the growth substances to increase the oxygen uptake of plants and tissues. Most of these studies were concerned with the exogenous respiration, the substrates (sucrose) being supplied in excess. It is obvious, that, when the latter is the case, the rate of oxygen consumption is controlled by the capacity of the respiratory enzyme system. The stimulative action, found in these experiments (COMMONER and THIMANN (1941), BERGER, SMITH and AVERY (1946), KELLY and AVERY (1949), ANKER (1951) et al.) was, therefore, undoubtedly exercised on the enzyme controlling the rate of the substrate oxydizing process.

In other investigations the above-mentioned problem was studied under entirely different conditions, namely of mild or strong starvation of the respiring tissues. Since in this case the substrate is limiting factor, it is evident that any stimulative effect under these conditions must be excercised on the substrate supply.

The present study belongs to the latter group of investigations, as the course of the respiration after I.A.A.-addition was studied with *starved Avena* coleoptile sections.

The information available from the literature shows, that no unanimity exists about the quantitative affectibility of the respiration when substrate is short. No stimulation was found by COMMONER and THIMANN (1941), BERGER, SMITH and AVERY (1946), whereas, even considerable increases of the endogenous respiration were found by BONNER (1949) and KELLY and AVERY (1949).

In the present investigation of the problem, with methods slightly different from those used by the authors mentioned-above, results were obtained which leave no doubt about a promoting influence of growth substances on the endogenous oxygen uptake.

A detailed study of the effect was made with sections cut from different positions on the coleoptile, after varying periods of starvation and in connection with the growth of the sections.

Next, the effect produced by other growth promoting substances was compared with that of I.A.A. and, finally, experiments were carried out concerning the influence of I.A.A. on starch hydrolysis by amylases *in vitro*.

For the explanation of the results, theories, based on the assumption that growth substances act on special enzymes or as components of enzymes, had to be discarded.

It is held, that the observed aspect of growth substance action is best explained by claiming a sensibilizing effect on inner lipophilic protoplasmic films, which, presumably, separate the enzymes and their endogenous substrates.

This view is a modification of the ideas of KONINGSBERGER (1942, 1947) and VELDSTRA (1944) who regarded only the external protoplasmic membrane as a lipophilic film.

CHAPTER I

MATERIAL AND METHODS

Seeds of *Avena sativa* (Victory Oat, Svalöv, 1949), after having been soaked in tap water for three hours, were sown on moist saw dust and grown in a dark room at 23° C and 90–96 % relative humidity. After 4 to 5 days the coleoptiles had reached the length required for manipulations. The coleoptiles were severed from the mesocotyl and about 5 mm were removed of the *lower* part, so that the primary leaves could be pulled out by hand. Next, the "empty" coleoptiles were divided into 3 mm sections with a cylinder microtome. The tips of the colcoptiles (4 mm) were rejected. Of each coleoptile three sections were cut. The sections of a given position were kept apart. Thus the influence of I.A.A. could be studied separately on the top sections (5–7 mm of the original coleoptile), the middle sections (8–10 mm) and the bottom sections (11–13 mm).

These sections were either used directly or after a 24 hours period of starvation. During starvation, they floated on buffer (KH_2PO_4, pH = 4.5) solutions after having been mounted on thin, massive glass rods. It appeared that by complete submersion of the sections the capacity of the respiratory enzyme system was reduced. Finally, the starved sections could be easily slipped into the respiratory vessels. Each vessel contained 30 sections.

The gas exchange was measured in Warburg respirometers. The speed of the rotating shaker was about 240 oscillations per minute. The sections were suspended in KH_2PO_4-solutions, the concentration of which will be given with the experiments. For CO_2-absorption, the central well contained 0.25 ml of a 10 %KOH-solution. By addition of filter paper the absorbing surface was enlarged.

Before adding the growth substance to the experimental vessels, the rates of oxygen uptake in control and experimental vessels were compared for two hours. In the ideal case these rates are equal. The actual differences, however, due to the natural variability of the material, were not negligible as is shown by the last column of Table I.

TABLE I

Comparison of the oxygen uptake in three vessels (a, b and c) containing 30 sections from the same position of the original coleoptile (5–7 mm).

Experiment	mm^3 O_2/hour			maximal difference in % of the lowest value
	a	b	c	
1	21.1	22.0	20.5	7
2	25.2	26.2	30.3	20
3	17.6	18.7	17.8	6
4	23.1	22.9	23.5	3
5	20.1	21.7	19.8	10
6	19.7	20.4	19.0	7
7	26.0	25.8	24.9	4
8	17.1	18.6	17.3	9
9	22.7	22.2	21.3	7
10	21.3	22.6	22.4	5

These differences in the initial rate of O_2-uptake were taken into account when the percentages stimulation or inhibition by a given growth substance were computed.

In order to be able to add the growth substances, the vessels were detached from the manometers. Care was taken that no liquid from the thermostat could enter the vessels (wiping with cotton wool). 0.5 ml of a growth substance solution (concentration five times that of the desired final concentration) was pipetted in the vessel making the total volume of the medium 2.5 ml. To the control vessels the same amount of destilled water was added.

This way of adding was preferred to tipping the growth substance in from the side vessel. For, quantitative transfer requires that the side-arms are washed with some fluid from the main compartment. In doing so, however, the sections get stuck at the walls of the main vessel or of the side-arm and it takes a long time of manipulation, before they are all back in the liquid, which is important for obtaining uniform conditions.

The growth substance solutions were prepared with distilled hot water and not by means of the addition of some ethanol. In the present experiments on the respiration of starved tissue it was not allowed, if ever, to use ethanol because this substance stimulates the respiration, as will be seen later.

During the whole experiment no white light was admitted to the sections. The readings were done in phototropically inactive orange light at 25° C.

No special care has been taken to avoid bacterial contamination. Only once bacteria were observed under the microscope at the close

of these long experiments. Here the oxygen uptake started to increase considerably after 7 hours and reached, within 10 hours, a level which was 3 times that of the normal amount of oxygen uptake per hour. The medium became turbid and many rod-shaped bacteria showed up under the microscope. The increase in oxygen uptake, when bacteria were present, was not followed by a decline, which appeared in cases where no contamination was found (see p. 29).

CHAPTER II

THE RESPIRATION OF THE AVENA COLEOPTILE

§ 1. Introduction

A considerable amount of research has been done on the respiration of the *Avena* coleoptile (see Bonner, 1948) and it has become clear that the biochemical mechanism is not different from that of other plant tissues. Measurements of the respiratory quotient of freshly cut coleoptile sections showed a value of approximately one, which is indicative for carbohydrate consumption.

Relatively few data are available concerning the *rate* of the respiration of this tissue, and in particular by which factors the rate is controlled.

This question was first studied by Bottelier (1939). When adding 2.5 and 5 percent of glucose to freshly cut sections, this author observed a considerable increase in the respiration, which led him to the conclusion that, normally, the substrates are the limiting factor in the process.

Bonner (1948) too, found that addition of 1 percent of sucrose caused 22 percent increase in the oxygen uptake of freshly cut sections. In later experiments (Bonner, 1949) the same sucrose concentration caused no increase in respiration. Here acids of the Krebs-cycle caused an appreciable response, which did not occur in the former experiments.

These results, when taken together, indicate that in the coleoptile sections the substrate concentration is or soon becomes the limiting factor in the respiration.

Since the interpretation of auxin effects on the respiration of coleoptile sections, suspended in buffer without substrate, made it necessary to settle this point, the experiments on this subject were repeated.

Next, the course of the respiration of coleoptile sections was studied during a period of 24 hours, also, when suspended in a substrate-free medium. These experiments were also preliminary to the study of the effect of growth substances on the respiration after varying periods of starvation.

§ 2. Experiments concerning the course of the endogenous respiration

Five-days-old coleoptiles were divided in 3 mm sections which represented the 5–7 mm, the 8–10 mm and the 11–13 mm of the

original coleoptile. The respiration of sections of a given position was studied separately. The experiments lasted from 4 p.m. to 4 p.m. or later on the next day. Interim opening of the vessels did not influence the respiration so that the oxygen pressure apparently remained above the critical value. In fig. 1 a few examples are given, taken from three different experiments.

The initial decline in respiration in the first hours is in agreement with the results of BOTTELIER and of BONNER. Rapid, as well as slow, declines were found. However, when the experiments were continued over a longer period, it appeared that, after about 12 hours, the gradual reduction in the respiration was followed by an increase in

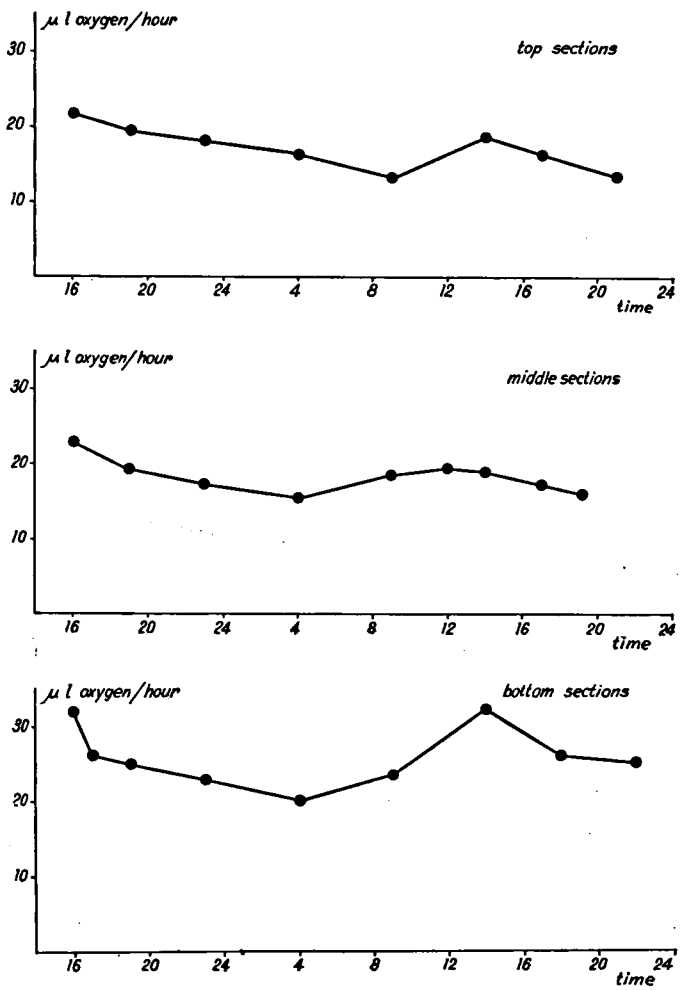

Fig. 1. The course of the endogenous respiration in *Avena* coleoptile sections, suspended in a substrate-free buffer solution. (three experiments)

the oxygen uptake, which, after another 10–12 hours, was followed by a new decline. This periodicity in the rate of the respiration was observable in all eight experiments, though, in some cases, it was not so evident as in the examples of fig. 1.

As a rule, the maxima on the second day reached about the level of the initial respiration rate. In this respect the bottom sections behaved differently, as the maximum on the second day surmounted the initial value. In fig. 4, p. 41, the courses of the endogenous respiration of top-, middle- and bottom sections of one experiment are comparable. Here too, the bottom sections reached a higher level on the second day. The curves of the oxygen values of sections of different levels of the coleoptile were not principally different and, generally, they ran parallel.

§ 3. Addition of glucose to starved coleoptile sections

It has been shown in the previous section that the intensity of the endogenous respiration varies periodically. These variations may, theoretically, be due to (1) variations in the activity of the oxydizing enzymes or to (2) variations in the intensity of the substrate mobilisation. The experiments of this section resulted in favour of the second possibility.

Sections of five-days-old coleoptiles were starved for 24 hours in the way described on p. 26. After the sections had been introduced into the 2 % KH_2PO_4-buffer, the rate of oxygen uptake was measured for 3 hours. Next, glucose was added to the final concentration of 0.5 %, after which the rate of oxygen uptake in the exogenous respiration was measured for another 3 hours. The quotients $\frac{\text{exogenous}}{\text{endogenous}}$ respiration of 7 experiments are summarized in Table II.

TABLE II

Quotients of $\frac{\text{exogenous}}{\text{endogenous}}$ respiration

Exp.	top sections	middle sections	bottom sections
1	1.95	2.43	2.43
2	2.08	1.81	1.98
3	1.65	2.17	2.36
4	2.10	2.04	2.08
5	1.88	2.13	1.88
6	1.40	1.64	1.64
7	1.81	2.02	1.83
Average	1.84	2.04	2.03

It appears from this Table that after adding glucose the respiration of the sections of all positions was about doubled, though the actual quotients varied from 2.43 (exp. 1) to 1.40 (exp. 6).

In these experiments the respiration was not measured during the preceding 24 hours period of starvation. It is therefore not known to what extent this process had been changed as compared to the respi-

ration of freshly cut sections. Still an estimation is possible, since the rates of oxygen uptake before adding glucose were of the same order as those mentioned in the experiments in the previous section. Here, after 24 hours, the average respiration had decreased about 10 % as compared with the initial value. Because of the fluctuations this average has to be accepted with proper reserve as appears from fig. 1c, where instead of a reduction an increase was observable after 24 hours. The conclusion is allowed, however, that the addition of glucose raised the respiration to a level which was much higher than that of freshly cut sections. This means, that (1) the respiratory enzymes were not much — if at all — harmed by the starvation and (2) that the respiratory rate of sections suspended in buffer is limited by the concentration of available substrate.

§ 4. Discussion

The experiments described above are an indirect confirmation of Bottelier's work, and therefore, they support the view that in starved as well as in normal coleoptiles the respiratory enzymes are not saturated with substrate.

The rate of the endogenous respiration appears to be about half of that of the exogenous one.

Ultimately the reserve polysaccharides are the substrate for the endogenous respiration, which, according to our own estimations, were found to be present as starch in large amounts, even after long periods of starvation.

The special suitability of starch to be used in the respiration has been emphasized by James (1946) in a review on the respiration of plants. The small difference in energy content between the glucose – glucose linkage and that of the phosphate-glucose linkage is the reason, that far less energy is needed to form the Cori-ester from starch than to transform free hexoses into phosphate esters.

Still more evidence is produced by R.Q. measurements on the basis of which Bonner (1948) presumed that the normal endogenous substrate is of a carbohydrate nature.

The fluctuations found in the intensity of the endogenous respiration remind one of the work of Bünning (1939). This author observed in photoperiodically active plants an endogenous rhythm in the activity of some processes. In the so-called night-phase many plants showed increased activity of the starch-hydrolyzing enzymes, which was accompanied by formation of free sugars and by an increased oxygen uptake. The rhythm in the oxygen uptake by the coleoptile of *Avena sativa*, which is a long day plant, might be of a similar character.

The maxima, however, were generally found in the early afternoon and not during the night. The cause of this divergence might be the differing external conditions. In the present experiments the seedlings were grown under constant conditions (darkness, constant temperature and humidity). The endogenous rhythm was, therefore, not affected

by the diurnal fluctuations of these factors, which presumably rule the "endogenous" rhythm under more normal conditions.

In the light of BÜNNING's work, the present fluctuations of the oxygen uptake become a strong indication that the rate of carbohydrate mobilisation determines the rate of the respiration in starved *Avena* coleoptile sections.

CHAPTER III

STIMULATION OF THE ENDOGENOUS RESPIRATION BY INDOLE ACETIC ACID AFTER A LONG PERIOD OF STARVATION

§ 1. INTRODUCTION

The first to study the effect of growth substances on the respiration of *Avena* coleoptile sections was J. BONNER in 1933. When adding a purified extract from the fungus *Rhizopus suinus* in different concentrations to sections which were cut 7 hours before, he observed an increase of about 20 %. This degree of stimulation was maintained for at least 4 hours. Thereupon a decrease to about the level of the control was observed, which took place within one or two hours.

In a second paper on this subject BONNER (1936) ascribed the above-mentioned effect to associated impurities in the extract of the fungus since he found no effect of pure auxins on the oxygen uptake in his new experiments.

In a third paper, BONNER (1949) returned to his original opinion as this time I.A.A. was found to enhance the oxygen uptake of *Avena* coleoptile sections both in the presence and in the absence of sugars in the medium. He states, however, not to have succeeded in finding an explanation for these varying results.

As for the influence of I.A.A. on the *endogenous* respiration of the sections, the next two papers confirm the negative results of BONNER's second paper.

The first came from COMMONER and THIMANN (1941), who obtained an increase in the respiration with various concentrations of I.A.A. only when sugar was present in the medium; with sections, respiring in water, no significant change in the O_2-uptake was brought about by I.A.A.

The same has been reported by BERGER, SMITH and AVERY (1946). I.A.A., added to sections in water, generally did not increase the respiration.

The main part of the present work is dedicated to this problem, which may be formulated as follows: do growth substances of the auxin type stimulate the aerobic respiration of *Avena* coleoptile sections when the substrate is the limiting factor in the process?

For this purpose sections were kept in a substrate free medium during 24 hours in the way described on p. 26. It was assumed that

after this period the respiration had become entirely dependent on the reserve carbohydrates and that the residual growth substance was about exhausted.

§ 2. Addition of I.A.A. to starved coleoptile sections

The experiments of this section have already been published in a preliminary note (Anker, 1951).

Sections from different levels on the coleoptile purposely were studied separately, whereas the cited authors mixed them. The I.A.A. was added after 24 hours to the final concentration of 1 mg/l. The results are summarized in the Tables III, IV and V and in Fig. 2.

a. The variability of the respiration

Before dealing with the influence of I.A.A. on the O_2-uptake, the attention may be drawn to Table III, where the respiratory rates of the controls of all (12) experiments are put together. This Table, when read in horizontal direction gives an impression of the wide variations of the respiratory intensity from day to day.

A very low rate was observed in exp. 1. This was, however, caused by the complete submersion of the sections during the preceding period of starvation. The lower rates in the experiments 10 and 11 are, presumably, due to the low buffer concentration (0.01 % KH_2PO_4) used. For the rest the influence of the buffer concentration on the intensity of the respiration, could not be made clear by these experiments because of the wide variations in the oxygen uptake in experiments at the same buffer concentration.

No influence of age and length of the coleoptiles, of which the sections were cut, on the respiration could be established with certainty.

Another type of variability is met with when Table III is read in vertical direction. As a rule, the rates of respiration in the top sections were exceeded by those of sections from lower positions on the coleoptile, the middle sections being intermediary. Deviations from this rule were found in exp. 6 (middle sections) and expts. 8 and 9 (bottom sections). The degree of increase in the respiration of the coleoptile in basal direction is shown in Table III by the quotient:

$$\frac{\text{respiration of bottom sections}}{\text{respiration of top sections}}.$$

This quotient, varying too, could not be correlated with length and age, nor with the respiration rates of the coleoptiles.

b. The variability of the effect of I.A.A. on the respiration

The effect of 1 mg/l I.A.A. on the respiration of starved sections of 3 different positions on the coleoptile was studied in 12 experiments. In 28 out of 36 (12×3) cases a stimulation was found, in one case no effect, whereas in 7 cases a slight inhibition of the O_2-uptake to a maximum of 6 % occurred. There was a great variability in the degree of stimulation (Table IV). In 20 out of 28 cases the percentage

TABLE III

The endogenous respiration of sections of different position on the coleoptile

No.	1	2	3	4	5	6	7	8	9	10	11	12
age [1]	4	4	4	4	4	5	4	5	4	4	5	4
length [2]	—	—	s	m	l	l	l	l	s	m	s	l
buffer [3]	2.5	2.5	2.5	2.5	2.0	2.5	2.0	2.0	2.0	0.01	0.01	1.0
top [4]	5.0 [7]	21.5	27.0	21.8	17.6	24.2	17.1	19.0	15.8	12.6	10.6	17.2
middle [5]	6.3	25.0	37.5	27.7	23.6	22.3	18.9	14.4	20.5	14.9	10.8	17.7
bottom [6]	8.3	28.4	48.8	36.0	30.0	25.6	22.6	14.0	20.2	19.2	13.3	19.1
bottom / top	1.7	1.3	1.8	1.6	1.7	1.1	1.3	0.7	1.3	1.5	1.3	1.1

[1] age of the coleoptile when sectioned, in days; [2] length of the coleoptiles: s = short = ca. 2 cm, m = medium = ca. 3 cm, l = long = ca. 4 cm; [3] concentration in % KH_2PO_4; [4] top sections = 5–7th mm of the coleoptile; [5] middle sections =8–10th mm of the coleoptile; [6] bottom sections = 11–13th mm of the coleoptile; [7] respiration in μl oxygen per hour.

TABLE IV

The percent increase of the endogenous respiration after addition of 1 mg I.A.A. per liter

No. / duration in hours	1 6	2 3.5	3 4.5	4 5.5	5 6	6 4.5	7 2	8 4.5	9 3.5	10 3	11 3	12 6	average stimulation
top	+ 12	— 3	+ 44	+ 5	+ 15	— 2	+ 12	+ 17	+ 7	+ 15	+ 30	+ 12	+ 14
middle	+ 30	— 6	+ 18	0	+ 13	+ 17	+ 3	— 3	+ 3	+ 24	+ 45	+ 21	+ 14
bottom	+ 8	+ 9	+ 11	— 5	— 6	+ 28	— 4	+ 15	+ 8	+ 17	+ 17	+ 1	+ 8

TABLE V

The effect of I.A.A. on the endogenous respiration and its distribution in the coleoptile.

	stimulation				No effect	inhibition max. 6 %
	30 %	20–30 %	10–20 %	0–10 %		
top	1	1	6	2	–	2
middle . . .	1	3	3	2	1	2
bottom . . .	0	1	4	4	–	3
total. . . .	2	5	13	8	1	7

exceeded 10 %; in 7 cases an increase of over 20 % of the respiration was found (Table V). In some experiments the response to I.A.A. was low (Table IV, exp. 2, 4, 9), whereas in others the percentage of stimulation in all sections was high (exp. 3, 10 and 11). In a number of other experiments fairly high stimulations in sections of one level were accompanied by inhibition of the oxygen uptake in sections of another level (exp. 5 and 8).

From these results it is not possible to derive a conclusive answer to the question, in which zone of the coleoptile the respiration is most sensitive to I.A.A. It seems to be located in the region of the top and middle sections, though in three cases it is found in the bottom sections. This variation in the location of the maximal response cannot be correlated with age, length and intensity of respiration or growth of the coleoptile (for the growth rate see p. 51).

Nothing can be said here about the duration of the effect of I.A.A. on the endogenous respiration since, even at the end of the longest experiments an apparent difference in the respiration rates is observable. The percent effect is generally constant during the whole observation period, though a slight decrease at the end of the experiment may occur.

In Fig. 2 the course of the respiration in experimental and control

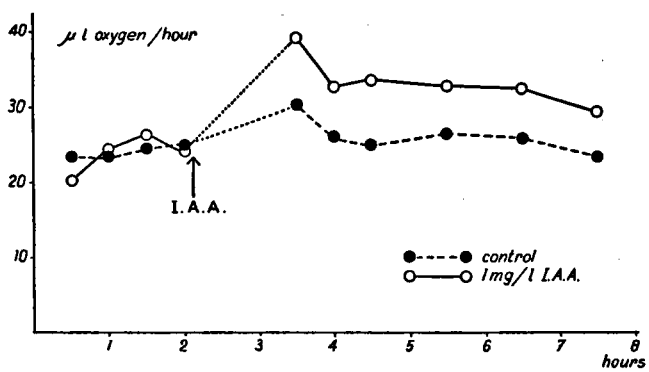

Fig. 2. The effect of I.A.A. on the rate of the endogenous respiration (experiment 6, bottom sections).

vessels is shown (exp. 6, bottom sections). Before I.A.A.-addition the amounts of oxygen taken up per hour were about equal; after the addition the respiration of the treated sections increased up to a level which was continuously about 25 % higher than that of the untreated ones.

In the next chapter the duration and other details of the effect will be described.

§ 3. Discussion

When searching for an explanation of the quantitative differences in the oxygen consumption from day to day, the differences in the size and shape of the coleoptile are, possibly, of primary importance. By factors, not yet under control, the coleoptiles vary from slender on one day to robust on another. Consequently, differing amounts of tissue are present in the respiratory vessels, which may largely account for the above variations. Aeration during the preceding starvation period and the concentration of the buffer solution are other factors determining the rate of the oxygen uptake.

Also the increase in the respiration in the basal direction of the coleoptile will be largely due to the fact that these organs end conically, the bottom sections being wider than those nearer to the tip. Parenthetically it is remarked that the zone of maximal growth certainly does not show the highest rate of respiration. The differences in diameter of top and bottom sections as well as the degree in variability of the diameters of the coleoptiles are shown in Table VI.

TABLE VI

Comparison of the diameters of top and bottom sections

Exp.		1	2	3	4	5	6	7	8
top sections	a	77	77	77	77	76	75	75	72
	b	70	70	70	70	65	62	62	62
bottomsections	a	88	88	88	86	86	85	84	84
	b	79	78	78	76	76	75	75	73

a = mean diameter in scale units in the direction through the vascular bundles; b = ⊥ a

Observations of J. Bonner (1936) on the distribution of the respiration over the length of the coleoptile are in favour of the view that in the present experiments the oxygen uptake is determined by the quantity of tissue since in his experiments the respiration, calculated per mg coleoptile, did not change significantly from the 5th to the 13th mm of the coleoptile.

Besides the morphological differences, physiological factors may have influenced the ratio of the oxygen uptake in top and bottom sections. The respiratory activity per mg protein, which, according to J. Bonner (1936) decreases in basal direction may be one of these. Taken by itself, this decrease is an indirect indication of increasing substrate shortage in the basal direction, since the enzymes are unsaturated. Microscopical estimation of the starch content on different

levels of the coleoptile by the present author, though pointing in this direction, is considered insufficiently exact to support this supposition. Differences in the activity or the amount of starch mobilizing enzymes may, theoretically, also play a part in determining the intensity of the respiration at different levels.

The rate of the respiration and of the I.A.A.-increased respiration is not connected with the growth of the sections. This will be shown in Chapter VI, see p.51.

There is much variability in the reaction of the respiration to addition of I.A.A., (1) in the quantity and (2) in the localisation of the effect.

Generally the endogenous respiration is stimulated by I.A.A., such at variance with the conclusion which COMMONER and THIMANN and BERGER, SMITH and AVERY have drawn from their experiments. This controversy may partly be explained by differences in the method. The sections taken by these authors from different levels on the coleoptile were thoroughly mixed, while in the present experiments sections from a certain position were studied separately. Application of their method would also have resulted in none, or an insignificant increase in the expts 2, 4 and 7. The great increase found in other experiments (3, 10 and 11), however, are unaccountable by this methodological difference.

The small effect in the experiments 2, 4, 7 and 9 is possibly due to the fact that in some cases the reactivity of the cells to I.A.A. was about annihilated by the relatively long starvation period. In experiments reported in the next Chapter the starvation period was reduced to a few hours. Without any exception, a considerable increase in the oxygen uptake was found with this material.

These results, therefore, confirm BONNER's positive results.

With 2,4-dichlorophenoxy-acetic-acid (2,4-D), a chemical with auxin activity, similar results were obtained by SMITH (1948). After treatment of bean seedlings with this substance a strong stimulation of the oxygen uptake (2.5 to 4 times the corresponding rates in the untreated slices) was observed on the subsequent days in the slices of the first internodal tissue.

KELLY and AVERY (1949), using *Avena* coleoptile sections, after a starvation period of 18 hours in distilled water, by which the respiration was reduced to about two third of the initial one, obtained stimulation of the oxygen uptake after addition of 2,4-D. The extent of the increase was dependent on the concentration used. With 1 mg/l stimulation of 10 to 20 % was found, which equals that in the present experiments with 1 mg/l I.A.A. Their results with *Pisum* in principle correspond with those reported for *Avena*.

These data from the literature, together with our own observations justify the conclusion that stimulation of the endogenous respiration is a real aspect of growth substance action.

Besides the variations in the quantity of the effect, differences are

found in the locus of maximal effect. The conclusion that this is generally within the first 10 mm of the coleoptile seems permissable. No correlation can be found between the place of maximal effect and such factors as age, length, respiration etc. of the coleoptile. To be able to determine whether these variations are due to a shift of the zone of maximal sensitivity to auxin, experiments with various concentrations of I.A.A. are needed.

The great variability of the *Avena* coleoptile in its reaction to added growth substances is not limited to the respiratory response. The growth response too was found to vary in such a way that results obtained on different occasions were hardly comparable (THIMANN and SCHNEIDER, 1938; SCHNEIDER, 1938 and BENTLEY, 1950). The last named authors stress the necessity of avoiding comparison of growth reactions of sections taken from different positions on the coleoptile.

CHAPTER IV

STIMULATION OF THE ENDOGENOUS RESPIRATION BY INDOLE ACETIC ACID AFTER A SHORT PERIOD OF STARVATION

§ 1. INTRODUCTION

In this Chapter experiments concerning the effect described in the previous one are put together. They were carried out with freshly cut sections, the I.A.A. being added within three hours after sectioning (during the first hours after sectioning the relative intensities of the oxygen uptake of the sections in experimental and control vessels were determined; see p.27). The main purpose was to determine the duration of the effect and, since for this reason the respiration had to be followed over a much longer period, it was deemed better to use fresh sections in order to avoid complications by possible degenerations of the tissues, as well as to reduce the risk of contamination. Attention was paid not only to the duration of the effect but also to the first and to the last hours of its appearance. Finally, the possibility was investigated, whether the limited duration of the effect was due to depletion or inactivation of the I.A.A. or to other factors.

§ 2. THE INITIAL EFFECT

Even in experiments in which I.A.A. strongly stimulated the respiration (when the total oxygen consumption in treated and untreated sections was compared) the effect was often negative during the first hour. The initial course of the respiration, however, differed according to the position of the sections on the coleoptile.

Of ten experiments, in which the effect was on the whole greatly promoting in all sections, the type of the effect during the first hours is given in Table VII. It appears that the stimulative action of I.A.A.

starts sooner in the middle sections than in the top and bottom sections and is mostly not preceded by a period of inhibition.

TABLE VII

The initial effect of I.A.A. on the endogenous respiration of sections from different position on the coleoptile (10 experiments)

time after addition	top sections		middle sections		bottom sections	
	+	—	+	—	+	—
60 minutes	3	7	9	1	2	8
90 minutes	6	4	9	1	7	3
120 minutes	10	0	10	0	9	1

+ = stimulation
— = no effect or inhibition

§ 3. The level of the I.A.A.-increased respiration

As a rule — it has been shown earlier — the respiration of freshly cut sections, being put in buffer solutions without substrates, declines

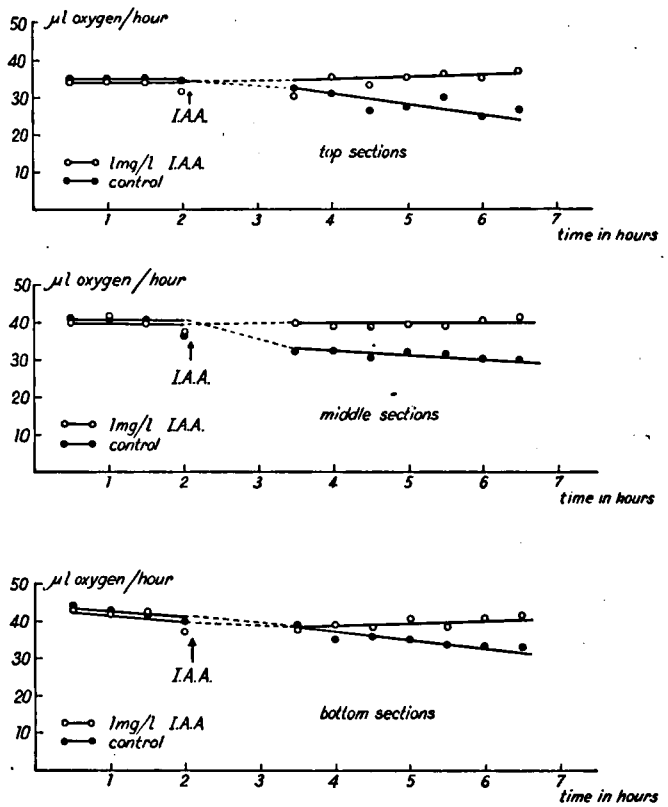

Fig. 3. The rate of the endogenous respiration is maintained on the level of that of freshly-cut sections by addition of I.A.A.

gradually with time. This decline starts either immediately or only after one or two hours. In one experiment sections of different position behaved differently in this respect. This is illustrated by Fig. 3, where the top and middle sections maintained a constant level of oxygen uptake during the first hours, whereas in the bottom sections the respiration declined from the very beginning.

This figure further shows the fact, already mentioned in the previous section, that the stimulation by I.A.A. was first observed in the middle sections.

The main reason, however, why this experiment is mentioned is to illustrate that the initial level of the respiration may be maintained for many hours when I.A.A. has been added. The effect of I.A.A. may therefore be described in this and other cases as delaying the decline of the respiration.

A similar observation was made by MICHEL (1951). In his experiments the oxygen uptake of hypocotyl sections of the Kidney bean declined rapidly in the controls, whereas the original value was maintained when I.A.A. was added previously.

§ 4. THE DURATION OF THE EFFECT

In nine experiments the respiration of untreated and I.A.A.-treated sections was followed over a period of 30 hours in order to investigate the duration of the stimulation.

This turned out to be as variable as are the quantity and the localisation of it. The average effect lasts nearly 20 hours in all sections. The results of six experiments with a 1 mg/l concentration and of three with other concentrations of I.A.A. are shown in Table VIII. It is clear from the detailed data (see below) that no much theoretical value should be attached to the averages.

TABLE VIII

Duration of the effect in hours

Exp.	concentration	top sections	middle sections	bottom sections
1	0.25 mg/l I.A.A..	21	14	14
2	1 mg/l I.A.A..	16	15	9
3	1 mg/l I.A.A..	3	25	25
4	0.50 mg/l I.A.A..	20	24	22
5	2 mg/l I.A.A..	21	16	19
6	1 mg/l I.A.A..	23	13	14
7	1 mg/l I.A.A..	30	24	27
8	1 mg/l I.A.A..	23	22	29
9	1 mg/l I.A.A..	17	12	17
	Average	19	18	19

Variations were also observed in one and the same experiment. Long-lasting effects in the top sections were accompanied by short stimulations in the other sections and reversely.

One of the causes ending the effect was a rise in the respiration of the untreated sections to the level of that of the treated ones. This is illustrated in Fig. 4. When comparing in this Figure the respiration rates in top, middle and bottom sections of the experimental vessels,

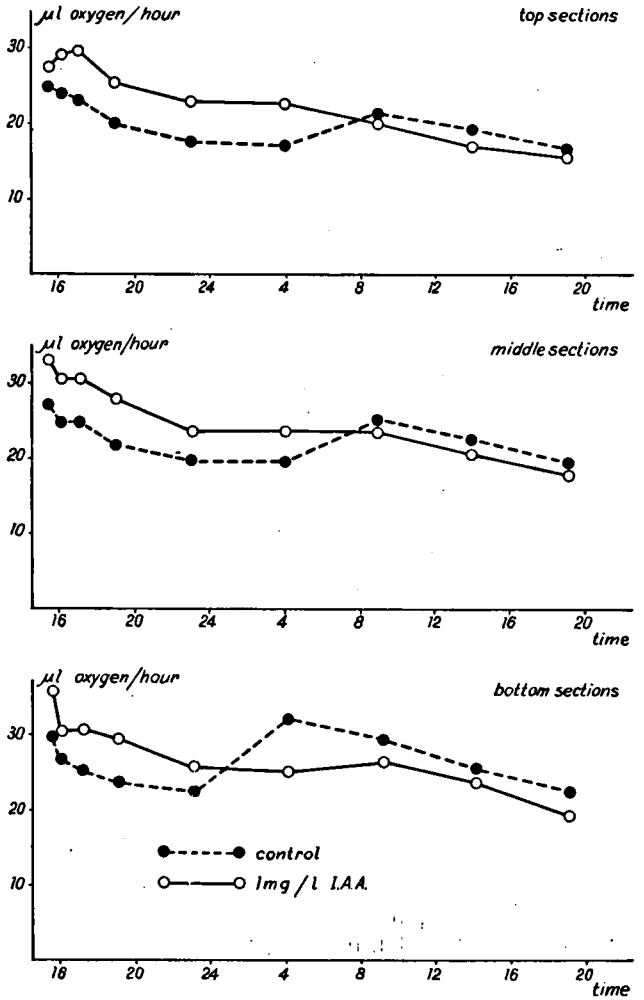

Fig. 4. The stimulation of the endogenous respiration caused by a 1 mg/l I.A.A.-concentration.

one observes that these do not fluctuate and run about parallel. As this is not so with the respiration in the control vessel, the effect is considerably curtailed in the bottom sections, since the increase in the oxygen uptake in these sections occurs much sooner than in the top and middle sections.

Further it is notable that the phenomenon, described in the second Chapter (p. 28) as an endogenous rhythm in the intensity of the respiration only occurs in the untreated sections.

In other experiments the effect ends more "normally" by a gradual decrease in the respiration of the I.A.A.-treated sections to the level of that in the untreated ones. This is shown in Figure 5, where ex-

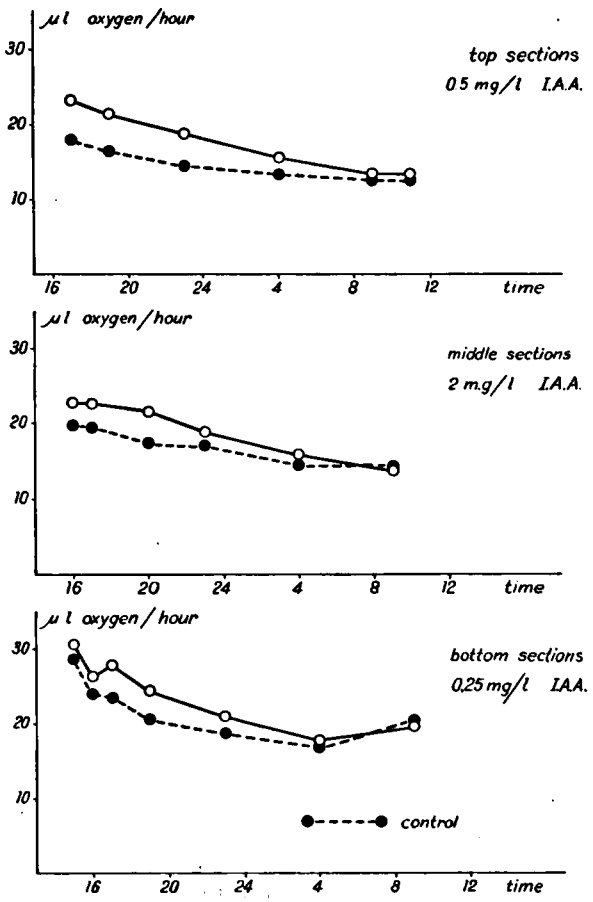

Fig. 5. The stimulation of the endogenous respiration caused by I.A.A. in the concentrations of 0.25, 0.5 and 2 mg/l.

amples, selected from three experiments with different I.A.A. concentrations (0.25, 0.5 and 2.5 mg/l) are given.

§ 5. The quantity of the effect

In the previous Chapter experiments were described concerning the influence of I.A.A. on the respiration of sections after a 24 hours' period of starvation. The results varied much in a quantitative sense.

Even in some cases no or a negative effect was observed. Much smaller variations were observed in the present experiments where I.A.A. was added within three hours after sectioning (see Table IX).

TABLE IX

Percentage of stimulation of the endogenous respiration of *Avena* coleoptile sections during the first 12 hours of observation.

Exp.	concentration	top sections	middle sections	bottom sections
1	1 mg/l I.A.A..	+ 33	+ 24	+ 5
2	1 mg/l I.A.A..	— 4 [1]	+ 11	+ 28
3	0.25 mg/l I.A.A..	+ 15	+ 13	+ 12
4	2 mg/l I.A.A..	+ 33	+ 12	+ 14
5	0.50 mg/l I.A.A..	+ 26	+ 23	+ 17
6	1 mg/l I.A.A..	+ 33	+ 22	+ 30
7	1 mg/l I.A.A..	+ 29	+ 27	+ 25
8	1 mg/l I.A.A..	+ 25	+ 11	+ 26
9	1 mg/l I.A.A..	+ 38	+ 42	+ 34
	Average	+ 25	+ 21	+ 21

[1] During the first hours the effect was strongly positive; by an uncommonly large increase in the respiration of the control sections, however, the effect became on the whole slightly negative.

Whereas it was concluded in the previous Chapter that stimulation occurred "generally", in these experiments the effect was always present. The average increase during the first 12 hours of observation even exceeded 20 % in all sections.

In total the extra oxygen uptake in the treated sections amounted to 100–150 μl, when I.A.A. was present in the concentration of 1 mg/l.

§ 6. Different concentrations of I.A.A.

No special experiments were carried out to gain information of the I.A.A. concentration which causes the maximal stimulation of the respiration. Usually, it was added in the final concentration of 1 mg/l. Only three other concentrations were used, each in one experiment (2 mg/l, 0.5 mg/l and 0.25 mg/l).

The preliminary impression was obtained that the I.A.A. concentration does not affect the duration of the effect, i.e. the stimulation was not prolonged or shortened by increasing or decreasing the I.A.A. concentrations respectively (see Table IX).

From these single experiments at best the preliminary conclusion may be drawn, that the 1 mg/l concentration was not far from optimal.

§ 7. Second addition of I.A.A.

In 6 experiments new I.A.A. was added to the sections at the moment that the effect of the first portion in at least two of three vessels had decreased to zero. So a final concentration of I.A.A. of nearly 2 mg per liter would have been obtained if no I.A.A. had been consumed or inactivated during the previous hours. Subsequent measurements

of the oxygen uptake during at least 6 hours showed that the effect of the new I.A.A. on this process was negligible. Immediately after the addition, in some cases fluctuations of the O_2-uptake were observed; within one or two hours, however, the course of the respiration continued in the original direction as if nothing had happened in between. The average effect of the second addition of I.A.A. did not surpass 1 %, which is insignificant.

§ 8. The influence of ethanol on the oxygen uptake

Up to a few years ago in the Utrecht Botanical Laboratory the I.A.A.-solutions were generally prepared by means of ethanol [1] which was evaporated afterwards. Complete removal of the ethanol, however, cannot be obtained by this method. The remaining negligible small amounts were thought to have no influence on the results when the growth of coleoptiles and coleoptile sections was being measured.

At the present time, the use of alcohol is avoided in our laboratory since the method of solving the crystals in hot water proved not to affect the activity of the growth substance molecules.

It is not known which method is used in other laboratories. In those papers on the influence of growth promoting substances on the respiration, cited in the present publication, no information is given about the procedure.

Apart from a probable influence of small amounts of ethanol on the permeability of the protoplasmic membrane, many cells — among which those of the *Avena* coleoptile (Bonner (1948)) — are able to use this substance as a substrate for the aerobic respiration. Hence, in studies on metabolic changes produced by growth substances, only the second method of preparing the solutions may be applied.

Kelly and Avery (1949) studied the influence of small amounts of ethanol on the respiration of *Avena* coleoptile sections in the presence of abundant sucrose (1 %). Even in this medium an additional increase in the oxygen uptake of 5–15 % was observed. Their experiments do not prove, however, that the increase caused by ethanol was due to an aerobic consumption of this substance since a stimulative effect on sucrose metabolism is not excluded.

A similar restriction holds true for the experiments presented in this section, in which the effect of ethanol on the endogenous respiration and on the I.A.A.-increased endogenous respiration was studied.

In these experiments with starved coleoptiles, which lasted 7 hours, after addition of 40 mg ethanol per liter, an increase in the oxygen uptake over the control of 8–23 % was obtained. In the experiment in which the highest increase was found (23 %), the extra oxygen uptake amounted to 26 μl, which is less than one fifth of the oxygen needed for the complete oxydation of the alcohol present in the vessels. So, for the case that the alcohol is consumed by the cells, the relative small increases point to a small capacity of the alcohol dehydrogenating system, since, under the same conditions, a 100 % increase in

[1] 0.5 ml per 10 ml distilled water for 10 mg I.A.A.

the oxygen consumption was found after addition of glucose (see Chapter II, p. 30).

The following observations were made (Fig. 6) on the influence of ethanol on the increase in the endogenous respiration by I.A.A. At the start of the experiment, ethanol reduced the positive effect of I.A.A. on the endogenous oxygen uptake to about zero. In the next one or two hours, the inhibiting effect of ethanol gradually disappeared so that in the following period no much difference was observable between the quantities of stimulation in the presence or absence of alcohol. In another experiment, however, the final increase in the oxygen uptake caused by I.A.A. in the presence of alcohol was higher than that of I.A.A. alone.

For this reason the question whether the actions of I.A.A. and ethanol on the respiration are independent, additive or even synergistic is hard to answer as the interaction of these substances — if present at all — seems to change in the course of an experiment.

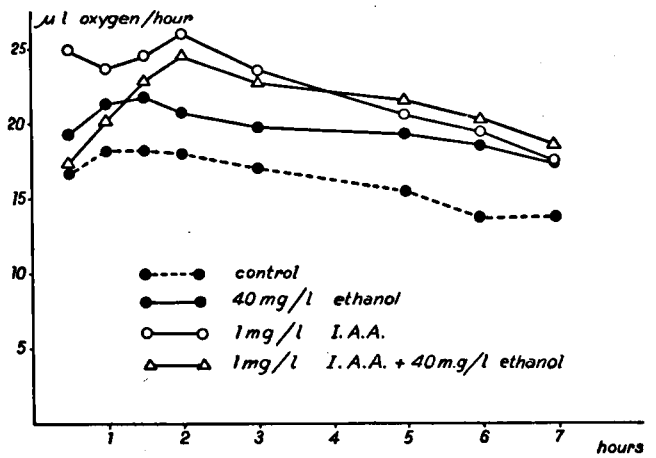

Fig. 6. The effect of ethanol on the respiration and on the I.A.A.-induced increase in the respiration, studied with sections suspended in a substrate-free medium.

It may be remarked, finally, that fig. 6 shows how, in experiments of short duration, the stimulative influence of I.A.A. on the respiration may be masked by the presence of ethanol in the medium. This might provide another explanation why other authors did not find any influence of I.A.A. on the endogenous respiration.

§ 9. Discussion

The experiments of this Chapter leave no doubt about a promoting effect of I.A.A. on the endogenous respiration of *Avena* coleoptile sections. The much smaller effects described in the preceding Chapter, when I.A.A. was added not until 24 hours of starvation had passed, are probably due to long-term unphysiological conditions which presumably reduce the reactivity and increase the variability.

To the same cause it is ascribed that the stimulative effect of I.A.A. is of a limited duration. Higher concentrations do not prolong the effect, more dilute solutions do not shorten it. Nor did addition of new I.A.A. cause a second stimulation of the respiration. These facts strongly indicate that consumption or inactivation of the growth substance do not determine the duration of the effect.

Another potential cause of the ending of the effect is the depletion of the reserve carbohydrates. In both, treated and untreated sections, however, only a small decrease in the starch content could be observed under the microscope when compared with freshly cut sections. In addition, the rate of the endogenous respiration was not much reduced at the close of these long experiments, so that exhaustion of substrate may be excluded too. Consequently, a limited reactivity of the cells is considered the factor determining the duration of the effect.

The stimulation was generally preceded by a varying period of inhibition. The same sequence of effects was observed by STENLID (1949) when studying the influence of the 2,4-dichlorophenoxy-acetic acid methyl ester on the respiration of wheat roots at pH 7.0, the period of inhibition lasting about 4 hours. This author observed with I.A.A. an inhibition of the respiration at pH 4.6 whereas at pH 7.0 a small stimulation was found after 30–90 minutes, which increased during the subsequent hours.

Significant differences in the reactions of sections of different levels on the coleoptile were not found, except for the observation that the promoting influence of I.A.A. started sooner in the middle sections. It would be of interest to investigate whether the growth response of sections of this level also starts earlier. This suggestion, however, is not based on the belief that the increased oxygen uptake is the result of increased growth, since simultaneous measurements of the growth and the respiration, to be reported in Chapter VI, p. 51 showed that stimulation of the respiration occurs independently of growth reactions.

CHAPTER V

THE EFFECT OF OTHER GROWTH PROMOTING SUBTANCES ON THE ENDOGENOUS RESPIRATION

§ 1. INTRODUCTION

In the experiments reported here, the actions of other auxin-like substances were tested in comparison with I.A.A. The effect on the endogenous respiration was followed during 5 to 8 hours with freshly cut sections, representing the 5–7 mm of the coleoptiles, the growth substances being added 3–4 hours after sectioning. As usual, the first hours after sectioning were used to compare the respiration in experimental and control vessels.

The substances tested were: γ-phenyl butyric acid, indole butyric acid, α-naphthyl acetic acid and β-naphthyl acetic acid [1].

The concentrations of the applied growth regulators were equimolar to a 1 mg/l I.A.A. solution.

§ 2. THE EFFECTS OF GAMMA-PHENYL BUTYRIC ACID, INDOLE BUTYRIC ACID, ALPHA-NAPHTHYL ACETIC ACID AND BETA-NAPHTHYL ACETIC ACID

The results of the experiments carried out with γ-phenyl butyric acid and with indole butyric acid are given in Table X.

TABLE X

Comparison of the percent stimulation of the endogenous respiration caused by indole acetic acid, γ-phenyl butyric acid and indole butyric acid.

Exp. no.	I.A.A.	γ-phen. but. acid	ind. but. acid	duration in hours
1	19	12	7	5
2	20	14	8	5
3	24	11	— [1]	8

[1] = not measured

In Fig. 7 the first experiment is presented.

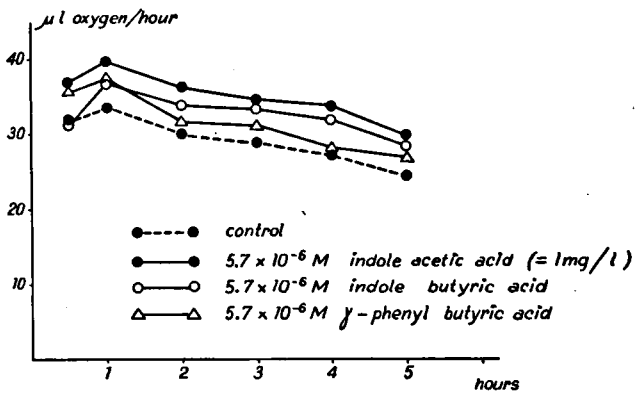

Fig. 7. The effects of indole acetic acid, gamma phenyl butyric acid, and indole butyric acid on the endogenous respiration of sections suspended in a buffer solution.

The results of the experiments with α- and β-naphthyl acetic acid are given in Table XI and in Fig. 8.

[1] The testing of the last substance was suggested by Dr H. Veldstra. For this, and for providing a sample of this substance the author is indebted to him.

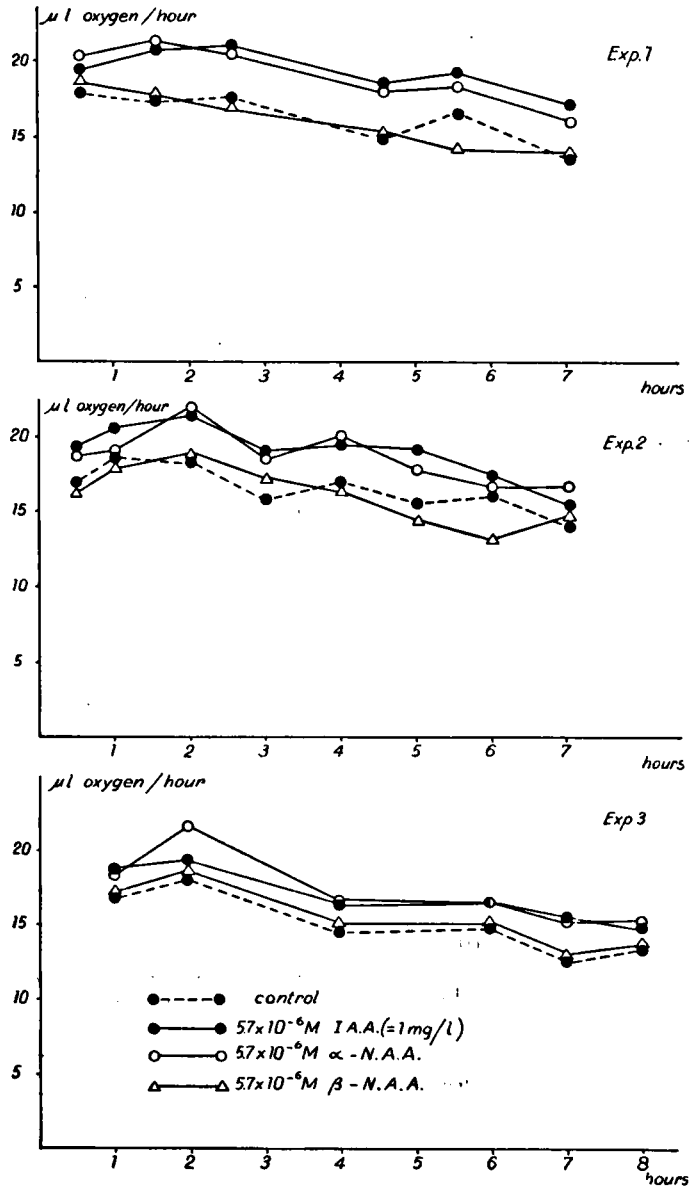

Fig. 8. The effects of indole acetic acid, alpha-naphthyl acetic acid, and beta-naphthyl acetic acid on the endogenous respiration of sections suspended in a buffer solution.

TABLE XI

Comparison of the percent stimulation of the endogenous respiration caused by indole acetic acid, α-naphthyl acetic acid and β-naphthyl acetic acid.

Exp. no.	I.A.A.	α-naphth. ac. acid	β-naphth. ac. acid	duration in hours
1	18	17	— 2	7
2	16	15	— 2	5
3	12	15	+ 4	7

§ 3. Discussion

γ-phenyl butyric acid

According to Went (1939), γ-phenyl butyric acid belongs to a group of substances, which may induce the "preparatory" reaction only and not the growth reaction proper. It is supposed to prepare a physiological condition within the tissue, which causes the effect of certain amounts of I.A.A. on the growth to be larger than on unprepared tissues. γ-phenyl butyric acid, therefore, is one of the so-called hemi-auxins which change the sensitivity of growing cells to auxin. Since the sensitivity of the cells is ascribed by Went to the food factor content, hemi-auxins are thought to be active on the food factor complex. One component of this complex was identified as sugar (Schneider (1938)).

Though it is obvious from Went's experiments that the effect of γ-phenyl butyric acid is not restricted to an action on the sugar supply, it is held that in the present stimulation of the endogenous respiration, this peculiar effect is probably the cause of the phenomenon observed.

The molar activity appears to be about half that of I.A.A.

Indole butyric acid

Contrary to γ-phenyl butyric acid, this substance is active in the growth reaction proper. Except for the standard *Avena* test, the molar activity is practically equal to that of I.A.A. (see Went, 1939–a). The relatively low activity in the standard *Avena* test shows that it lacks one or more secondary properties, since these are supposed to influence the quantity of the curvature.

In the present experiments it appears that the property of activating the endogenous respiration was present to a lesser degree than in I.A.A. and even in the "hemi-auxin" γ-phenyl butyric acid.

α-naphthyl acetic acid

Unlike the former, this substance was equally active in the respiration as I.A.A. Still, its activity in the standard *Avena* test does not differ very much from that of indole butyric acid. This means that, in the case of α-naphthyl acetic acid, inferiority in the standard *Avena* test is caused by another secondary property than that manifesting itself in the present experiments.

Further research along this line with a number of other growth promoting substances will perhaps help in future to clear up the mechanism of action of a given substance on a given process.

β-naphthyl acetic acid

This substance did not stimulate the endogenous respiration. VELDSTRA (1944) found that the effect on the root formation was about one third of that of the alpha compound and half that of I.A.A.

ZIMMERMANN and WILCOXON (1935) observed a small activity in causing sweet Pea stems to bend. The alpha compound appeared to be approximately 100 times more active than the beta. They did not exclude the possibility that this small activity was even due to slight contamination with the alpha compound. The present results add to the probability of this supposition.

The importance of testing growth substances on the respiration as a selective test for their activity is shown by the absence of such an effect of beta-naphthyl-acetic acid.

CHAPTER VI

RESPIRATION AND GROWTH

§ 1. INTRODUCTION AND METHODS

Earlier in this paper (p. 33) the attention has been drawn to the variability in the response of the endogenous respiration to the addition of I.A.A. Similar quantitative variations were observed by other authors when they measured the growth response of *Avena* coleoptile sections.

The experiments reported here, in which the growth and the respiratory response were determined simultaneously, were designed to detect whether the magnitudes of stimulation of these processes are quantitatively related.

For this purpose plants were selected, of which the primary leaves were about piercing the coleoptiles. This was implied by the fact that in the greater part of the plants the primary leaves had already done so (such individuals were not used). The sections of these coleoptiles were expected to show only a small response to I.A.A.

A second group of experiments was carried out with sections taken from short coleoptiles, the growth response of which is generally larger than that of sections from long coleoptiles.

The growth measurements (determination of the final length of the sections) were carried out with the same sections of which the reaction of the respiration had been determined before. For reasons of comparison it was deemed more exact not to use sections staying under more normal conditions for the growth measurements. As abnormal conditions within the respiratory vessels are to be mentioned the absence of carbon dioxide in the atmosphere and the uninterrupted shaking. BÜNNING, HAAG and TIMMERMANN (1948) showed that

mechanical stimulations caused inhibition of the cell elongation with etiolated seedlings of *Sinapis alba* and of *Vicia faba.*

The sections, being freshly cut, were suspended in a 1 % KH_2PO_4 solution without addition of substrate.

The I.A.A. was added in the final concentration of 1 mg/l. The concentrations of the other growth substances were equimolar to that of I.A.A.

The respiration was measured over a period of 5 to 8 hours, at the end of which the final length of the sections was determined.

§ 2. Simultaneous measurements of growth and respiration

The results of these experiments are summarized in the Tables XII and XIII. The main conclusions are:

1. In experiments with sections taken from *long coleoptiles* the growth of the I.A.A.-treated ones is equal to, or slightly above that of the controls.

2. In all cases a stimulation of the oxygen uptake is found, the percentage of which was independent of the final length of the sections.

TABLE XII

The influence of growth substances on the endogenous respiration and on the growth of sections taken from long coleoptiles

Exp. no.	growth subst.	final length of the controls		final length of treated sections		% stimulation of the respiration
		M	E_M	M	E_M	
1	I.A.A.	56.9	0.6	56.9	0.5	24
2	I.A.A.	61.1	0.3	62.3	0.4	13
3	I.A.A.	58.6	0.3	60.5	0.5	35
4	I.A.A.	59.5	0.3	62.4	0.8	13
5	I.A.A.	59.0	0.5	60.3	0.4	20
	P.B.A.	59.0	0.5	59.1	0.3	14
	I.B.A.	59.0	0.5	59.8	0.7	8
6	I.A.A.	57.6	0.4	58.2	0.7	24
	P.B.A.	57.6	0.4	57.2	0.5	11
7	I.A.A.	60.3	0.5	60.3	0.5	12
	a-N.A.	60.3	0.5	60.2	0.3	15
	b-N.A.	60.3	0.5	59.5	0.5	4

The final length of the sections is expressed in scale units.
The original length amounted to 53 being 3 mm.
I.A.A. was present in the concentration of 1 mg/l, the concentration of the other growth substances being equimolar to this.
P.B.A. = γ-phenyl butyric acid
I.B.A. = indole butyric acid
a-N.A. = alpha naphthyl acetic acid
b-N.A. = beta naphthyl acetic acid
M = mean of 10 sections measured
E_M = standard error

From (1) and (2) it follows that the stimulation of the respiration occurs in the absence of increased growth.

3. In experiments with sections taken from *short coleoptiles* the

growth is increased in the presence of I.A.A., alpha-naphthyl acetic acid and indole butyric acid.

4. The percent stimulations of the respiration are not larger than those observed in non-growing sections of long coleoptiles.

TABLE XIII

The influence of growth substances on the endogenous respiration and on the growth of sections taken from short coleoptiles.

Exp. no.	growth subst.	final length of the controls		final length of treated sections		% stimulation of the respiration
		M	E_M	M	E_M	
1	I.A.A.	58.3	0.7	63.9	0.5	31
2	I.A.A.	56.6	0.2	64.5	0.5	11
3	I.A.A.	60.3	0.3	61.9	0.6	19
4	I.A.A.	57.9	0.5	61.0	0.8	18
	a-N.A.	57.9	0.5	60.2	0.7	17
	a-N.A.	57.9	0.5	56.1	0.4	— 2
5	I.A.A.	60.3	0.5	67.3	0.8	19
	P.B.A.	60.3	0.5	61.6	0.7	12
	I.B.A.	60.3	0.5	66.9	1.0	7

For explanation see under Table XII.

From (1), (2), (3) and (4) it follows that actual growth does not correlate with, in any case does not increase, the stimulation of the respiration.

The percent elongation of the sections from short coleoptiles after addition of I.A.A. varied from 15–25 percent in 5–8 hours.

§ 3. Discussion

The increase in length of 15–25 percent indicates that the conditions for section growth were not extremely bad within the respiratory vessels, since Rietsema (1950), who used the normal cylinder test method with sections suspended in a 0.01 m KH_2PO_4 solution in the presence of 0.1 mg/l I.A.A. observed an elongation of 25–30 percent during the same period of observation. As, however, the concentrations both of the buffer- and of the auxin solutions differed from those used in the present investigation, the results are not quite comparable.

On an average, the stimulations of the oxygen uptake of the growing and non-growing sections were equal. This excludes that the effect of growth substances on the respiration is only the result of the elongation of the sections.

Blank and Frey Wyssling (1941) discovered that an impressive synthesis of protoplasm takes place in Mais coleoptiles during cell elongation. It is an open question whether this part of the growth process can be influenced independently. In the present experiments it is, theoretically, possible that in both growing and non-growing cells the same amounts of new protoplasm (including phosphorylative (?) enzymes) are formed after I.A.A. addition, such at the cost of the reserve nitrogen in the vacuole (see Blank and Frey Wyssling

(1940)). This could account for the fact that the magnitude of stimulation of the endogenous respiration does not depend on the occurrence of growth.

The results, described earlier, however, present evidence that the observed increase in the oxygen uptake is not due to the formation of new enzymes. In several experiments it was found that after the period of stimulation the rates of oxygen uptake in the treated and the untreated sections showed only minute differences or were equal again. This seems to point to a temporary — direct or indirect — stimulation by I.A.A. of the activity of enzymes already present, rather than to a promotion of the formation of new enzymes.

CHAPTER VII

THE INFLUENCE OF INDOLE ACETIC ACID ON THE AMYLASE ACTIVITY IN VITRO

§ 1. Introduction

The experiments reported in this section are a repetition of part of the work of Eyster (1946), Smith, Langeland and Stotz (1947) and Brakke and Nickell (1952). These authors obtained contradictory results when studying the influence of I.A.A. and of other growth substances on the activity of isolated diastases.

In measuring the diastatic activity two procedures can be followed. First, the amount of unchanged starch can be measured at regular intervals by using the iodine staining technique. Secondly, the amount of reducing sugars, formed in this reaction can be determined by titrating the reaction products.

The first method was used by Eyster who measured the time required for the digestion of soluble starch past the last iodine staining stage. All growth regulators appeared to inhibit the diastatic activity, the effect being correlated with the pH of the medium that was not buffered.

Smith, Langeland and Stotz determined the effect of I.A.A. on the diastatic activity by measuring the saccharification rates using the alcaline ferricyanide technique, described by Redfern and Johnston (1938). No stimulation or inhibition was observed.

After the experiments reported in this chapter had been completed, a paper was published by Brakke and Nickell (1952), who studied the influence of growth regulators of different structure on the activity of an alpha amylase secreted by virus tumor tissue from roots of *Rumex acetosa* grown in vitro. The enzyme activity was determined spectrophotometrically, by measuring the decrease in optical density of the starch iodine complex. The experiments were made with different growth substance concentrations but in no case a significant effect was obtained.

In the present investigation both the iodine staining technique and the sugar titration method were tested. It appeared that the first

method was less suitable in this special case, since in the presence of I.A.A. complications occur which may easily lead to incorrect interpretations.

§ 2. The iodine staining technique

To one of two series of Pyrex test tubes containing buffered solutions of 1 % amylum solubile (2½ % KH_2PO_4, pH = 4.5) plus pure alpha and beta amylases (5 mg/l), I.A.A. was added to the final concentration of 10 mg/l. To the other series distilled water was added instead. The progress of the reaction was studied at 22° C. through adding every half hour 0.1 ml of a fivefold diluted Will's solution (6 g KJ + + 2 g J_2 in 120 ml aq. dest.), which caused a blue colour of the test fluid. The rate of starch hydrolysis was measured by determining the time required to get "no blue colour" after addition of iodine, since "no blue colour" means, that all starch has been broken down to final or intermediate products. At the eighth observation (after 4 hours) this occurred in experimental as well as in control tubes, a fact indicating that the reaction rate was not much affected by I.A.A., though — it is admitted — the intervals between measurements were rather long.

The fact that the "no colour" point was reached by experimental and control tubes simultaneously was surprising, since observations during the previous hours seemed to point to an acceleration of the reaction by I.A.A. From the beginning the colour in the experimental tubes was of a less intensive blue than in the controls. As soon as it was noticed, however, that the colour of the experimental solution was not constant and even disappeared after about 8 hours, it became obvious that complications were going on. After adding fresh iodine the colour reappeared.

It is common knowledge that the blue colour of a iodine-stained starch solution can be made to disappear by heating, and that after cooling the colour will return. With I.A.A. present in the solution in ample concentration, the blue colour did not return, the solution remained uncoloured. If, however, iodine was added in excess, the presence of I.A.A. did not make any difference.

This problem was solved by the following experiment. When a solution of I.A.A. + J_2KJ was heated to about boiling point the reaction mixture became turbid. This turbidity appeared at room temperature as well, but at a much slower rate. The precipitation was not analyzed. Addition of starch showed that iodine had disappeared from the medium. From this the conclusion was drawn that iodine had combined with I.A.A.

After these observations the explanation of the colour differences in experimental and control tubes is obvious. The extinction of the colour is due to the disappearence of free iodine from the reaction medium.

These experiments were not continued since, in the meantime, well reproducible results were obtained with the following method.

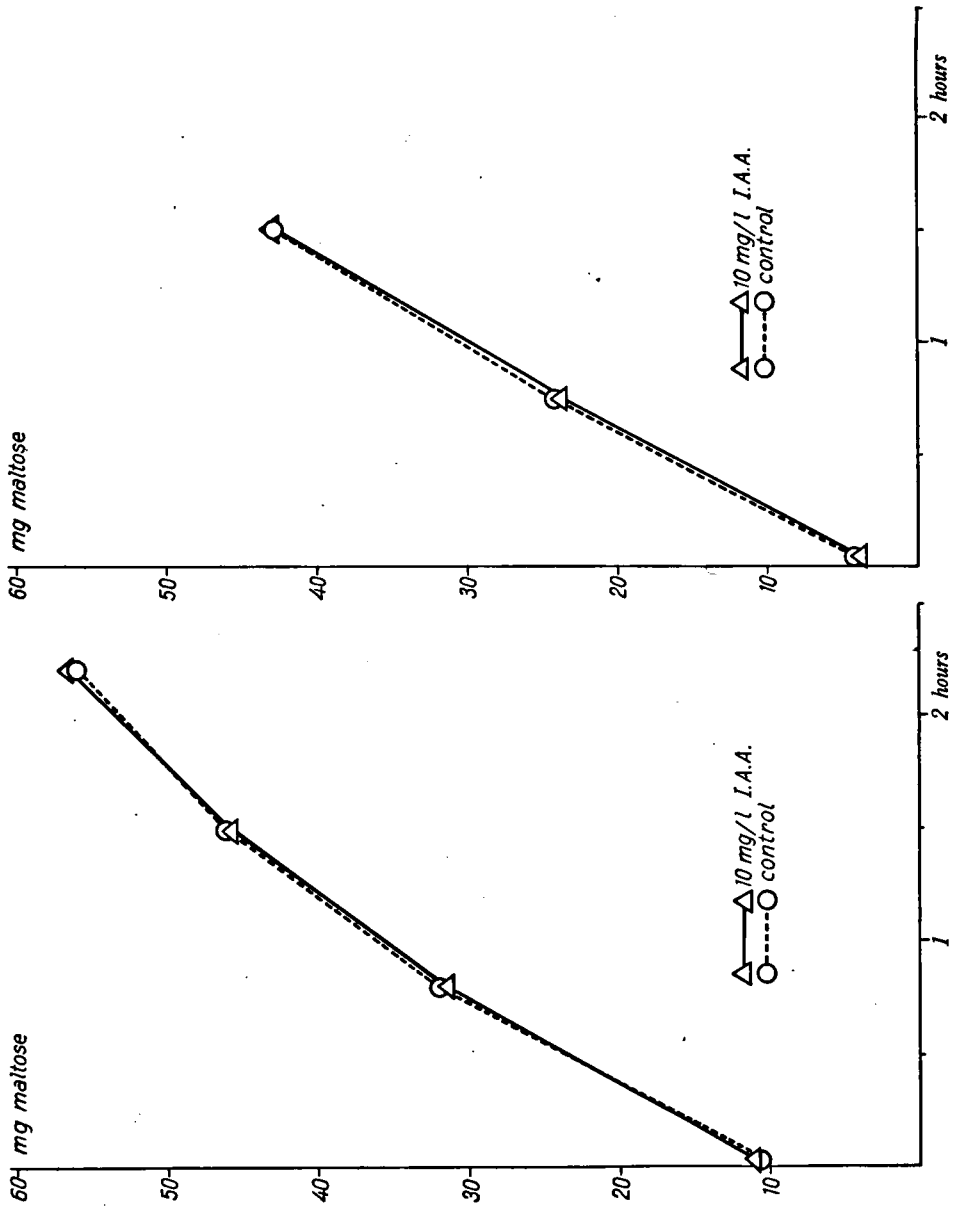

Fig. 9. Absence of effect of indole acetic acid on the amylase activity in vitro.

§ 3. Determination of sugar production

The titration was carried out after the method of Schoorl (1929). It is based on the reduction of cupri- to cupro ions by reducing sugars, the remaining cupri ions being determined iodometrically.

The diastatic activity was studied in a medium containing 0.75 % amylum solubile, 100 p.p.m. I.A.A. and 1–1.5 p.p.m. alpha and beta amylases. The reducing power of the reaction mixture is expressed in mg maltose.

The results show that I.A.A. does not influence the rate of starch hydrolysis by amylase in vitro (fig. 9).

§ 4. Discussion

It seems justified to conclude from the data presented in the literature, which are confirmed by the present experiments, that direct stimulation of the amylases is not the mechanism by which I.A.A. stimulates the starch hydrolysis. There is no special reason to assume that the results of these *in vitro* studies are of less importance for conclusions on the situation in the living cell than similar *in vitro* studies were with other enzymes, such as dehydrogenases etc.

The absence of a direct influence of I.A.A. on the amylases does not exclude a stimulation of amylolytic activity *in vivo* since it may be caused by other mechanisms, for instance, a release of an enzyme from a bound state to an active one. This point will be discussed in the next chapter.

That Eyster nor Brakke and Nickell did notice extinction of the blue colour in the presence of I.A.A. must probably be ascribed to the fact that more concentrated iodine solutions were used, or, that the solutions, after having been observed, were immediately rejected.

GENERAL DISCUSSION

§ 1. Did I.A.A. function as a substrate for the respiration?

Before dealing with the possible mechanism of the I.A.A.-induced increase in the oxygen uptake, the question must be discussed whether this effect might be explained by oxidation of the growth substance itself. Was the extra oxygen, taken up after addition of I.A.A. perhaps used — wholly or partly — in a "respiratory process" in which I.A.A. functioned as a substrate?

This is no purely theoretical possibility, since enzyme systems, which inactivate I.A.A. under oxygen consumption do exist (Tang and Bonner (1947), with etiolated pea epicotyls; Wagenknecht and Burris (1950), with bean roots). In this reaction one molecule of oxygen was taken up and one molecule of carbondioxyde was released per one molecule of transformed I.A.A. Since the end product of this breakdown could be solved in ether and since it had an intact indole nucleus, the latter investigators concluded that indole aldehyde might be the end product.

If the possibility is taken into account that such an enzyme system

also occurs in *Avena* coleoptiles, the question becomes important whether this oxydation might serve as an explanation for the extra oxygen consumption observed after addition of I.A.A. to the sections.

In the experiments described above the effect was caused by an I.A.A. concentration of 1 mg per liter. For oxidation of all molecules, 0.32 μl of oxygen would be needed per vessel. Since, on an average, in the vessels with I.A.A. 5 μl per hour more oxygen was taken up than in the control vessels, and since the stimulation could last more than 20 hours, it is obvious that oxidation of the I.A.A. — if occurring at all — could only account for a negligible part of the effect.

§ 2. Stimulation of the reserve food mobilization by I.A.A.

It has been shown in the second Chapter (p. 30) that (1) the rate of the endogenous respiration of *Avena* coleoptiles is about half that of the exogenous respiration and that (2) this relatively low oxygen uptake is not caused by a reduction in the capacity of the respiratory enzymes under the prevailing conditions of starvation. Since, even after long experiments, no exhaustion of the starch content was observed under the microscope, the conclusion has been drawn that the rate of the endogenous respiration is determined by the rate of reserve food mobilization.

A considerable number of investigations has shown that stimulation of this mobilization must be considered a normal aspect of growth substance action. (Borthwick, Hamner and Parker (1936), Mitchell and co-workers (1937, 1938, 1940, 1945) Reinders (1938, 1942) and many, more recent publications). The products originating from this process were thought to provide material for cell wall formation and for other synthetic growth processes, or to cause water uptake by enlarging the amount of osmotically active material.

Still another possibility was suggested by Reinders (1938, 1942). She observed an increased loss of dry weight when adding I.A.A. to discs of potato tubers. This effect was ascribed to an oxydative breakdown of the starch. Also Rasmussen (1947) suggested an increased utilisation of reserve carbohydrates in the respiration after addition of 2,4-D to roots of *Taraxacum officinale.*

In the author's opinion, the present results have to be explained by the same phenomenon, since the respiration was limited by the substrate concentration. When the latter is the case, no increase in the oxygen uptake may be expected by stimulation of the substrate oxydizing enzymes. The observed increase in the endogenous respiration cannot be explained, therefore, by the theory of Commoner and Thimann (1941, see p. 24), which was based on experiments with ample supply of substrate. It must be emphasized, however, that this inapplicability only concerns the bruto oxygen uptake in the present experiments and not possible qualitative changes in the endogenous respiration. These changes might occur in the *way* of the *consumption* of the mobilized reserve food (for instance a stimulation of the C_4-dicarboxylic cycle).

When the stimulative action of I.A.A. is interpreted as providing

substrates to the respiratory enzymes, the question arises by what mechanism the reserve carbohydrates are made more readily available to the respiration.

§ 3. The mechanism of the effect

If I.A.A. and other growth regulators really affect the starch consumption inside the cells, there are, apart from an improbable direct stimulation of the hydrolyzing enzymes, other possible mechanisms of action. One of these, proposed by Eyster, is a release of the enzymes from a bound state to an active one. This was suggested by results obtained with model experiments, in which growth regulators were found to release diastase from activated charcoal. Smith, Langeland and Stotz (1947), however, repeating this investigation under more rigorous experimental conditions were unable to confirm this result.

Another possibility is that starch inside the cell is surrounded by proteins, as by some investigators glycogen is supposed to be (Willstätter & Rhodewald (1934) and Przylecki & Majmin (1934)). In that case the increased consumption of starch by addition of I.A.A. might be explained by removing the hypothetical protective layer and thus making the starch more accessible to the diastases (see below). Such actions on proteins were already claimed by Northern (1942), who suggested as the primary action of auxins a dissociation of associated protein molecules, which would cause stimulation of diverse metabolic processes, including starch hydrolysis.

The above considerations on the stimulation of the starch breakdown by hydrolysis may be extended to the phosphorylative activity of the cells. These enzymes, which catalyze the combination between phosphoric acid and polysaccharides were found in many parts of higher plants (Hanes, 1940). They give rise to the formation of the Cori-ester (glucose-1-phosphate) which, in case of further oxydative breakdown, undergoes transphosphorylation to glucose-6-phosphate. It is held by Meyer (1943) that with glycogen this is the normal way of breakdown in the cell metabolism, such in contrast to what happens in the digestive tract, where it is attacked by amylases. Possibly, the same holds true for starch when it is involved in an oxydative breakdown.

In recent papers much attention has been paid to the possibility that the primary action of auxins is exercised on the phosphate metabolism.

Bonner and Bandurski (1952) are of the opinion that auxin "must in some way affect the phosphorylative process". Their view is partly based on the assumption that the rate of the respiration — which is increased by auxins — is limited by the phosphorylation and by neither the substrate concentration nor the capacity of the respiratory enzyme system.

With Bonner, Van Overbeek (1952) believes that the organic acid metabolism provides a substrate for auxin action, in the way that by this part of the respiration the high energy phosphates are generated "the transfer or utilisation of which is regulated by auxins".

Although phosphorylation of starch cannot occur without phosphates, it is held that the endogenous respiration is not stimulated by facilitating the phosphate supply. For, it cannot be assumed that in the present experiments with starved sections the phosphate concentration was controlling the rate of oxygen uptake at the moment of auxin addition. Addition of sugars being immediately followed by a rapid increase in the respiration, it is obvious that the amount of free sugars was limiting the rate of oxygen uptake before, and not those agents by which the sugars are involved in the oxydative breakdown (phosphates, phosphorylating and oxydizing enzymes).

At this point of the discussion an argument may be moved, derived from observations reported in Chapter IV § 2. (p. 38). It was found that the stimulative action of I.A.A. was preceded by a period of inhibition, which lasted some 1–2 hours. In terms of phosphate metabolism this would mean an I.A.A.-induced withdrawal of phosphates from the phosphorylative enzymes, the original situation being restored only after one or two hours.

It seems more obvious that in this initial inhibition of the respiration another link in the chain of endogenous respiration is affected by I.A.A. In this connection the attention may be drawn to papers of KONINGSBERGER (1942, 1947).

KONINGSBERGER and co-workers, using isolated protoplasts obtained from mesophyllous tissue of bulb scales of *Allium cepa*, studied microcinematographically the effect of I.A.A. on the change in volume when these protoplasts were transferred from a 1.5 M into a 0.75 M glucose solution. The increase in volume (i.e. the water uptake) was not only delayed for 7–9 minutes as compared with the control, but also markedly retarded over a period of at least 1–2 hours (fig. 10).

With the considerations of VELDSTRA (1944) in mind, these results

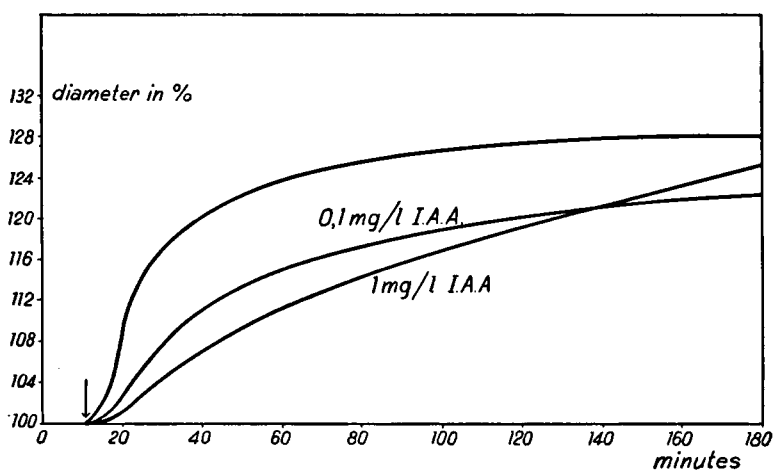

Fig. 10. The effect of indole acetic acid on the increase in volume of isolated protoplasts of *Allium cepa*, when transferred from a 1.5 mol/l to a 0.75 mol/l glucose solution. (after KONINGSBERGER, 1947).

were explained by claiming a condensing effect of I.A.A. on the protoplasmic membrane which caused a decreased water permeability. In some cases the final volume of the I.A.A.-treated protoplasts exceeded that of the controls, which pointed to a decrease in the elastic properties of the protoplasm.

Similar reactions of the protoplasm were described by NORTHEN (1942) as a decrease in the structural viscosity of the protoplasm due to the dissociation of cellular proteins.

The present effect of I.A.A. on the endogenous respiration runs more or less parallel with the results of KONINGSBERGER et al., a positive effect being often preceded by a 1–2 hours period of inhibition. The observed change in metabolic activity, therefore, might be explained in terms of variations in the structure of inner protoplasmic membranes, which increase or decrease the accessibility of enzymes to substrates (in casu starch).

This explanation is completely in agreement with the ideas of BÜNNING (1936) concerning the regulation of the respiratory intensity. BÜNNING, being convinced that the rate of the respiration in plants is, to a high degree, dependent on the substrate concentration, considered the reserve food mobilisation as the rate controlling process of the oxygen uptake. The amylolytic activity, in its turn, would depend on the presence or absence of a spatial separation between the starch and the enzymes. Evidence in favour of the existence of protoplasmic membranes between enzymes and substrates has been reviewed by VON PRZYLECKI (1935) for the case of polysaccharide breakdown.

A similar general influence and not an effect on a special enzyme was implied by STENLID (1949) when he explained the stimulation by 2,4-dinitrophenol on the respiration of wheat roots. As the mechanism of action he suggested a "destruction of some cell structures which leads to increased contact between some respiratory substrate and an enzyme system normally not working with full efficiency. It is known that many organs normally contain both enzymes and the appropriate substrates but that in spite of this no reaction occurs". It has been mentioned earlier that this author too observed a stimulation of the respiration after addition of the methyl ester of 2,4-dichlorophenoxy acetic acid, which was preceded by a period of inhibition.

It will be clear that the present experiments with *starved Avena* coleoptile sections were not intended to check the validity of the theories of THIMANN *et al.*, BONNER and BANDURSKI and of VAN OVERBEEK.

The above theoretical considerations, therefore, only mean to search for an explanation for those cases of stimulation of the respiration, in which the other theories do not seem applicable.

The fact that gamma phenyl butyric acid, a substance with a negligibly small effect on the growth reaction proper, is active on the endogenous respiration, proves that auxin action includes more than what has been studied here, and what might be best considered as an aspect of, what WENT called the preparatory reaction. (Chapter V, § 3, p. 49).

WENT (1939) believes that in the preparatory reaction growth substances have a positive effect on the upward transport of certain substances, needed for cell elongation (food factor).

In isolated sections, suspended in a substrate free medium, however, such factors cannot be supplied by transport. Consequently, their growth, which is considerably increased by I.A.A., is dependent on the reserve substances. From this it follows that in this case at least, part of the food factor complex is mobilized on the spot. Since, even in freshly cut sections the respiration is markedly increased after addition of sugar (which points to substrate shortage), it is not unlikely that the above also holds true for intact coleoptiles. By the presence of large quantities of starch this probability is still increased.

It may, therefore, be tentatively concluded that the preparatory action of growth substances is of a physico-chemical nature, being exercised on protoplasmic interfaces. It is not unlikely that also changes in such membranes may increase the upward transport of substances, needed for the growth of intact coleoptiles.

This is not the place for broad discussion on the probability that the growth reaction proper and other effects (increased cambium activity, root formation, etc.) are caused by the same mechanism. However, a few remarks may be made.

Since the manifold chemical reactions occurring in the hydrophylic protoplasm do not proceed chaotically, it is not improbable that the loci of these reactions are separated and that the reactions are regulated by structures which are of a different nature (lipophilic). As there is no reason for assuming that these lipophilic films are all of exactly the same structure, one may suppose that growth substances regulate the cell activity as a whole, as a consequence of different affinities to these films. According to this idea the effect of different concentrations of growth substances (optimum curve for each type of reaction as well as the manifold final effects, dependent on growth substances concentration) seems to become more easily conceivable.

The weakness of implying such general actions on the protoplasmic organisation is, however, that it is not sufficiently supported by clear-cut experimental evidence.

SUMMARY

1. By means of the Warburg manometric technique some details were studied of the respiration of starved *Avena* coleoptile tissue.

2. For this purpose the oxygen uptake was measured on 3 mm sections cut from different levels on the coleoptile (5–7, 8–10 and 11–13th mm from the tip). Instead of being thoroughly mixed — a procedure followed by other authors — the sections from a given position on the coleoptile were studied separately.

3. The amount of oxygen taken up per section per hour showed a slight increase in the basal direction of the coleoptile.

4. Addition of glucose to starved sections caused an increase in

the oxygen uptake of an average of one hundred percent. This is considered an indication that the respiration of such sections is limited by the substrate concentration.

5. The intensity of the endogenous respiration showed diurnal fluctuations. Arguments were moved to connect this with diurnal fluctuations in the rate of starch mobilization.

6. Addition of indole acetic acid (1 mg/l) to sections, starved over a period of 24 hours, generally caused an increase in the oxygen uptake.

7. The respiration of sections of different positions on the coleoptile showed quantitative differences in the response to indole acetic acid addition. The zone of maximal sensitivity, though generally situated between the 5–10 mm from the tip of the coleoptile, in some cases was found at the level of the lowest sections.

8. In experiments with freshly cut sections, the indole acetic acid being added within three hours after sectioning, without any exception, considerable stimulations of the oxygen uptake were found. The stimulations were preceded by a period of inhibition, the duration of which varied according to the position of the sections in the original coleoptile.

9. The stimulative effect of indole acetic acid on the oxygen uptake of freshly cut sections lasted nearly 20 hours on an average. The total extra oxygen uptake in the treated sections amounted to 100–150 mm^3, when indole acetic acid was present in the concentration of 1 mg per liter.

10. Addition of new indole acetic acid to the sections at the end of the period of stimulation was not followed by another increase in the oxygen uptake.

11. Addition of ethanol in a concentration as usually applied in dissolving growth substances (40 mg/l) increased the oxygen uptake of freshly cut sections by 8–23 percent. The presence of ethanol influenced the stimulation caused by indole acetic acid.

12. The action of other growth promoting substances was compared with that of indole acetic acid. Whereas the response of the endogenous oxygen uptake to alpha naphthyl acetic acid appeared to equal that of indole acetic acid, beta naphthyl acetic acid had no influence at all. The activity of gamma phenyl butyric acid being about half that of indole acetic acid, even exceeded that of indole butyric acid.

13. The degree of the stimulation in non-growing sections (cut from very long coleoptiles) was not less than in growing sections. The increase in oxygen uptake, therefore, was not the result of increased growth of the sections.

14. In accordance with data from the literature no effect of indole acetic acid was observed on the hydrolysis of starch by amylases *in vitro*. The unsuitability of the iodine staining technique for such investigations was demonstrated, as indole acetic acid combines with the iodine.

15. It is suggested that the auxin action, underlying the increased endogenous oxygen uptake, is exercised on inner protoplasmic mem-

branes (lipophilic interfaces) thus making the reserve food more accessible to the metabolic processes.

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