THE RELATION OF PROTOPLASMIC STREAMING IN THE *AVENA* COLEOPTILE TO RESPIRATION AND AUXIN TRANSPORT

By

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T A B L E O F C O N T E N T S

CHAPTER I

INTRODUCTION

Statement of the problem

One of the most remarkable features concerned with the various effects of auxin is found in its rapidity of transport through living cells from one part of the plant to another. This rapid rate of transport far exceeds that which can be accounted for by diffusion alone even in spite of the many structures (cell walls, plasma membranes, etc.) through which auxin must pass during its course. Another feature is that concerning the path of auxin transport. This is found to follow no specially adapted cells such as is found for the translocation of nutritive substances in vascular bundles, but may occur in parenchymatous tissues as well.

Several explanations have been offered to account for the mechanism involved in this type of transport but each has been criticized on the basis of inadequate proof. One of these, namely that which offers the streaming of protoplasm as a mechanism of transport is remarkably in accord with all the facts known to be associated with transport, as will be pointed out in the following paragraphs. Yet, more direct proof of this concept by demonstration of increased transport by a specific substance which also increases streaming is as yet lacking since the investigation concerning the effect of various substances

on both streaming and transport is meagre. The establishment of this proof was accepted as the aim of this investigation.

Since the auxin relations in the Avena coleoptile, the standard test object for auxin assay, are more completely worked out than for any other plant material this organ has been chosen as the subject of the investigation.

The protoplasmic streaming of the coleoptile has been shown by Bottelier (1935) to depend upon oxygen pressure, and has thereby been associated with respiration. Therefore, in searching for a substance that will increase the streaming rate it is more likely that such a substance will be found among those substances which, in general, increase respiration.

It is therefore that the effect of these substances on the rate of protoplasmic streaming is studied in Chapter II of this investigation. Unfortunately, no such protoplasmic streaming "accelerator" was found among these substances, but the results obtained led to the necessity of a study of their effect on respiration. Hence, in Chapter III the effect of some of the most effective substances was studied on the respiration of the coleoptile in an attempt to establish a closer link between protoplasmic streaming and respiration. In Chapter IV their effect is studied on auxin transport of the coleoptile in order to link together all three processes; streaming, respiration, and transport.

Literature)

The theory that protoplasmic streaming serves as a means of transporting organic materials was first put forth by de Vries (1885). Bierberg was the first to investigate this possibility by comparing transport of lithium nitrate in those cells of Vallisneria which exhibit streaming and those which do not. To account for the rapidity of the transmission of the phototropic stimulus Brauner, (1922) suggested that such a mechanism is also effective in the transport of growth regulators. This he based upon observation of a rapid streaming of the protoplasm in the cells of the coleoptile. Van der Wey (1932) questioned this, however, since he found the rate of transport to be independent of temperature, whereas protoplasmic streaming in most material increases in velocity with rising temperature. Bottelier (1934), however, found that in young coleoptile of Avena the protoplasmic streaming rate between 17° C and 35° C is little influenced by temperature. Hence the possibility still remained that auxin transport is controlled by streaming. Bottelier further studied the effect of external conditions on protoplasmic streaming in the coleoptile and demonstrated there a remarkable parallelism between the effect of light on the streaming velocity and the well-known light-growth reaction. The light-streaming reaction like the light-growth reaction was found to be proportional to the total energy and further

was found to show the same variation with the wave length of the light. Further, he showed that the so-called "standard" phenomenon in which the same auxin concentration produces a different curvature at different periods of day or month runs parallel with changes in the protoplasmic streaming.

Bottelier also sh owed that a decrease of O_2 pressure decreases the protoplasmic streaming. This is of interest in view of the data given by Van Ameyden (1917) on the effect of low oxygen pressure in decreasing growth and curvature.

Du Buy and Olson (1938 a,b) showed that an applied electric potential similarly effected, after about the same interval, protoplasmic streaming, transport, and growth, thereby further establishing the link between protoplasmic streaming and transport.

All of these experiments which connect the two processes, protoplasmic streaming and transport, are further supported by the work of Curtis and his school (1935, 1937) in their exhaustive reviews on the problems of translocation. After consideration of all the possible mechanisms, this author has concluded that only protoplasmic streaming as a means of transport can explain the data thus far available on translocation.

There are, of course, some objections to the explanation of transport by protoplasmic streaming. Van der Wey in 1932 found that the auxin transport in coleoptiles was strictly polar, from tip to base, and remained so even

against a concentration gradient. It is argued by some that protoplasmic streaming does not offer a means of maintaining this polarity. However, the protoplasmic streaming is responsible only for the intracellular transport from one end of the cell to the other, and the cell walls have to be traversed by other means. The polar characteristics of auxin transport are, in this theory, due to specific properties of these cell membranes. Another objection is that the transport of several substances other than auxin have been demonstrated to be independent of streaming. Kok (1931, 1932) failed to find any influence of protoplasmic streaming on the transport of lithium and of caffein in Vallisneria. Moreover, Schumacher (1936) using the fluorescence microscope observed that in the haircells of Cucurbita fluorescein moved strictly polarly towards the tip of the hairs. This movement is independent of the streaming and since the dye appears to move through the cross walls as readily as through the cells he suggests that the movement is in some way associated with the wall itself. Neither of these two contributions, however, are concerned with auxin nor are they concerned with plant material commonly used in transport experiments. Another conflicting bit of evidence is that provided by Clark (1938) in his study on the relation of electric potentials to transport. He reports that sodium glycocholate completely abolishes polar transport in the Avena coleoptile while it has no effect whatever on streaming. He also reports that

saponin completely stops streaming at a given concentration while it in no way affects transport. The effect of the sodium glycocholate does not conflict with evidence for transport by protoplasmic streaming since it could affect the membrane transport associated with the polarity of movement and thus stop transport and yet have no effect on the intracellular transport by protoplasmic streaming. The effect of the saponin, however, does conflict but it is discussed later in this investigation. It is seen, then, that there is no direct proof against the protoplasmic streaming-transport period with the exception of the inhibition by saponin.

Other theories of transport mechanism have been offered. Auxin has been found to be surface active, spreading itself in a mono-molecular layer. Kogl (1933) has suggested that auxin may be transported by its formation of a mono-molecular layer in a lipoid layer of protoplasm. Van den Honert (1932) has pointed out the possible importance of surface activity by which certain fluids can move along the interfaces between various substances. These hypotheses, however, are supported by no direct evidence and therefore as yet cannot be considered.

Electrophoresis has also been proposed as a mechanism of auxin transport. Since the active component auxin is considered to be the anion it would be expected to move toward positively charged regions. Moreover, studies on inherent

electric potentials or polarity of the Avena coleoptile showed the tip to be negative and the base positive under certain conditions. Went, (1932) based a theory of polar auxin transport upon these considerations and upon some experiments with various dyes. It was found that acid dyes move in the plant tissue more rapidly toward the base than toward the apex while basic dyes moved in the reverse direction. Several investigators: Waller (1929), Ramshorn (1934), Brauner (1935) have offered a further relationship between electrical polarity and auxin transport. This field has been reviewed in detail by Clark (1937) and will not be taken up here. Clark (1937, 1938) has investigated in detail the relationship between electric polarity and transport and finds these definite characteristics to be independent of each other. It is true that he found the effect of light on the inherent polarity of the coleoptile to be similar to the light-growth reaction but in no other case was there a similarity between the two processes. He suggests that electric polarity is in some way associated with membrane function and is in this way associated with polar transport but its activity is at present far too low to be measured. Therefore, electrophoresis as a mechanism of auxin transport can only be associated with that part of the translocation through living cells which is intercellular and thus dependent upon the properties of the cell membranes.

In conclusion: there exists no serious objection

against the theory that the protoplasmic streaming is responsible for that part of the translocation of substances which is intracellular.

CHAPTER II

FACTORS INFLUENCING PROTOPLASMIC STREAMING

METHODS

Improvement of the observation of streaming

The epidermal cells of the *Avena* coleoptile are long and cylindrical with somewhat pointed ends. The protoplasm appears somewhat granular under normal microscopic illumination, and it is the motion of these granules that is used as an index of the velocity of protoplasmic movement. The streaming follows slightly spiral paths along the wall in the direction of the long axis of the cells, but these paths lie in no orderly arrangement and appear to slowly shift in position from time to time. The width of these paths or strands may vary during the course of observation, but under constant conditions the average velocity of the granules or particles remains about the same.

In all previous investigations normal bright field illumination with red light (down to 5400 A°) has been used, the coleoptile being cut lengthwise and placed with the cut surface downward on an object slide under a cover glass. With such illumination, however, observation is far from favorable. Since the coleoptile even at its thinnest portion (between the two lateral vascular bundles) is five cell layers thick, the much shorter plastid-containing

parenchyma cells scatter and refract rays of the condenser cone to the extent that optimum resolving power is diminished. The particles appear as diminutive, gray, ill-defined points of varying degrees of size and intensity while the protoplasm itself can be differentiated from the vacuole as a grayish mass in which the visible particles are dispersed. In addition to the serious disadvantage of such a system in causing eyestrain after continued observation, particles from several microns in diameter down to the lower limit of visibility are visible, and the smaller the particle the greater its velocity. Previous investigators, in order to overcome this difficulty, have measured the velocity of the "fastest moving particles", and hence the smaller particles. However, the fastest moving particles cannot always be chosen and extreme variability of individual readings can occur. These disadvantages led to the following method of illumination in order to improve visibility for this investigation.

In a previous investigation (du Buy and Olson, 1938) infiltration of the coleoptile with paraffin oil not only sufficed to enhance the effect of applied electric potentials on the streaming by removing surface water films, but served also to diminish scattering of light (by filling intercellular air spaces) to the extent that the protoplasmic particles appeared as small, uniform, well-defined spheres. The smaller particles are no longer to be seen, but those visible are of constant size and therefore of a

more constant rate. This effect suggested that most of the difficulty of observation of the particles in coleoptiles immersed in water was due to the light-scattering capacity of the intercellular air spaces, and that the water infiltration of these would do much in enhancing the observation of uniform particles in the same way.

This was carried out by subjecting the immersed coleoptile to negative pressure in a vacuum dessicator repeatedly during several minutes. By such treatment most of the air in the intercellular spaces was displaced, but only after the coleoptile was allowed to remain under a cover glass for an hour or more was the observation improved significantly. Here uniform particles appeared but not as sharp and distinct as those made visible by oil infiltration.

Since dark field illumination increases the visibility of small particles whose refractive index varies but little from their dispersion medium, as in the study of certain microorganisms, it seemed that such a method of illumination would provide ideal contrast of the particles if scattering effects of cell wall irregularities, etc. could be excluded. However, the normal use of dark field illumination demands that the object viewed must consist of small units (microorganisms, colloids, etc.) in an optically clear medium, and its use in viewing particles in the protoplasm of the coleoptile would be complicated by refraction from the structures of underlying cells. This was found to be the case with all standard types of

dark field condensers. The light refracted into the objective from the tissue as a whole resulted in a brilliant glare which completely masked all cellular detail.

It was found, however, that if all the rays of light are masked out except those which approach the coleoptile in one direction parallel to its long axis this glare is obliterated. Furthermore, it was found that this unilateral type of dark field illumination under certain conditions of condenser distance, slide thickness, and degree of masking made visible the protoplasmic particles in brilliant contrast against a dark background as in the case of microorganisms with normal dark field illumination! (Fig. 1.) The cell wall outline also remained visible in less contrast, but no other structures complicated the field except occasional cross walls or groups of plastids in the underlying parenchyma cells. The effectiveness of this type of illumination is attributed to the narrow, converging cone of light which emerges from the periphery of the upper condenser surface at such an angle that it illuminates only the upper cell layer in the field of view. Since cross walls in this layer of long epidermal cells are not frequent the more highly refractive particles alone remain to divert the light into the objective and stand out in bright contrast against the dark background. So far as is known dark field illumination of this type has not been used before for several layered multicellular tissues.

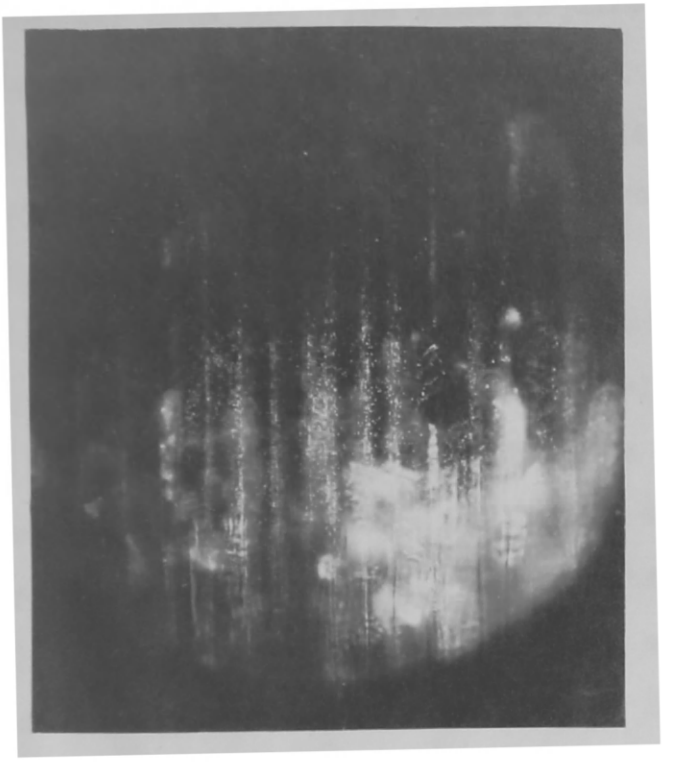

Fig. 1: Photomicrograph of the epidermal cells of the Avena coleoptile by unilateral dark field illumination. The low power used here shows the course of the condenser beam through the tissue.

The dark field illumination used in all the measurements in this investigation was obtained in this manner with a standard Abbe condenser through the use of a diaphragm below the condenser to cut out the objectionable rays of light. A 4 mm. objective with the usual funnel-stop for dark field inserted was used with a 10X Leitz micrometer occular. The time required for a particle to traverse a distance corresponding to 100 μ was measured with a stop watch, and the average of ten such readings during a two minute period is given for the rate for that period in terms of μ / second.

Since Bottelier (1934) has shown that the streaming velocity is influenced by phototropically active light a Corning "Yellow Shade Yellow" filter was used (transmitting down to 5460 A^{o}) with a 50 C.P. 6-8 volt microscope lamp adjusted for parallel illumination. A Zeiss water cell 5 cm. in thickness filled with a 2 per cent solution of $CuSO_4$ to which a few drops of H_2SO_4 were added was interposed to cut down heat radiation.

A photographic recording method

Even with such a method as the preceding one there still exists some variability between the rates of individual visible particles (since frequently some can be seen overtaking others). The measurements, then, are still somewhat subjective when concerned with small changes in the streaming rate, for it is quite possible to choose for

measurement, unknowingly in some cases, those particles which move at different rates from the average rate. In order to rule out this possibility a completely objective method based on photography was devised.

An illuminated particle when moving against a dark background should, when photographed during a measured interval, leave a streak on the film proportionate to the distance it traverses during that interval. Since there will be as many streaks on the film as there are particles moving in the field during that interval this should provide a means of recording accurately the average velocity of streaming at a given moment by averaging all streak lengths. A camera utilizing 35 mm. film was constructed and fitted to the Leitz "Micro-Ibso" attachment in order to measure velocities in this manner, and was found to be useful, but with definite limitations.

First: there was considerable overlapping of streaks in those regions where particles were moving close together. This could be prevented somewhat, however, by

(1) increasing the magnification thus further separating the particles, and by

(2) moving the film in a direction perpendicular to the streaming during the exposure.

The streaks would then follow the resultant of the two components of motion and the rates could be determined from

the component corresponding to the direction of the streaming.

Second: the intensity of illumination, although it appears brilliant to the eye, is not sufficient to record separate and distinct streaks during the short exposure needed when the magnification is high enough to limit the number of particles in the field.

The main difficulty, then, can be removed by increasing the intensity of the light source or by increasing the sensitivity of the film. The latter is more desirable in view of the sensitivity of the streaming to intense illumination. This might be done by sensitizing some of the newer ultra rapid films with ammonia just before use.

In view of the above complexities this method has been used only occasionally up to the present.

Material

Seeds of Avena sativa (var. Victory) were soaked for three hours and germinated on moist filter paper in the dark with occasional exposure to red light, at 23.5 ±.5° C. When the coleoptiles had reached a suitable length (4-5 cm.) they were removed from the seedling, cut lengthwise through the two lateral vascular bundles and infiltrated with water in a vacuum desiccator during several minutes. They were then placed under a cover glass, cut surface downward, and after an hour or more for complete infiltration and recovery from wound shock, were ready for observation.

Microthermostat and method of applying solutions

Since Bottelier (1934) has shown that any sudden change in temperature can inhibit and even stop streaming for a time, it was considered advisable to provide some means of accurate temperature control for both the observed coleoptile and for the solutions to be applied. Although the experiments were carried out in a dark room maintained at 23.5 $\pm$.5° C, additional and more accurate control was maintained through the use of a water microthermostat. Since the condenser of the microscope was water-immersed, a system was provided whereby this water between the object slide and the condenser could be circulated through a constant temperature bath. This was accomplished by constructing from hard rubber a special microscope stage in which the space above the condenser was closed in by a section of thin-walled rubber tubing (diameter 1½ inches). In this way the condenser was movable for adjustment, yet water from the bath could be forced under the object slide, above and around the condenser and back to the bath again. (Fig.21,22) The bath was maintained at 23.5 $\pm$.03° C and the water was circulated by a small circulation pump.

Bottelier (1935, p. 290) also showed that a coleoptile under cover glass soon takes up enough oxygen from the small volume of water there to limit the streaming rate. Therefore all solutions applied must be circulated or made to flow past the coleoptile in order to renew this oxygen

deficiency. In the following work the solutions were placed in one of two constant levelling vessels in the bath, and were led to the preparation through capillary tubes. The solution was allowed to drop from the ends of these tubes (thus becoming air saturated) to the object glass, where it was taken up under the cover glass. At the same time the solution already under the cover glass was continually siphoned away by a wick of glass cloth and carried down into a waste vessel (Fig.23,24). Since the flow in the capillary tubes could be controlled by screw clamps on short lengths of pure gum rubber tubing incorporated in the system, the rate of dropping could be adjusted to equal the rate of removal of solution. A continuous flow of a small and measurable amount of solution under the cover glass was thus maintained.

In experimental procedure the streaming rate was first studied in tap water until a constant rate was maintained over a period of fifteen minutes or more. The experimental solution was then introduced in place of tap water. All solutions were made up with tap water and were not applied until they had reached a temperature of $23.5 \pm .03^{\circ}$ C.

EXPERIMENTAL RESULTS

In order to speed up protoplasmic streaming a substance must either increase the "force" which effects

the motion or it must reduce the resistance to flow, or "viscosity", of the protoplasm. The former would appear to be linked with some metabolic process, whereas the factors governing the latter are little known. Therefore only those substances which might in some way increase the "force" contributing to the streaming are considered here.

The relationship between streaming and aerobic respiration strongly suggests that any factor which will increase the rate of the latter process should increase as well the rate of streaming. Such substances might react as direct accelerators of respiratory process or indirectly by influencing some other limiting factor. Therefore a study of the effect on streaming of those substances which were likely to affect respiration was made. As yet no substance has been shown to <u>increase</u> the rate of respiration in the oat coleoptile. Therefore the choice of substances for these experiments was based upon their known effects on respiration in other material. In many cases, interesting relationships between streaming, respiration and transport could be brought out, some of which have led to further work included in later chapters.

<u>Effect of anaesthetics on streaming</u>

It is well known that several anaesthetics such as ether, chloroform, etc. inhibit respiration in high concentration and in many cases accelerate respiration at very low concentration. (Irving, 1912; Thoday, 1913; Thomas, 1918; and others.) Therefore solutions of ether at different

degree of saturation were allowed to flow under the cover glass and the rates of streaming determined. Concentrations of ethyl ether solution above 32 g./liter of water caused an immediate stop of streaming, and inhibition was observed down to a concentration of 10 g./liter. Below this concentration no increase in streaming rate could be detected.

Chloroform and alcohol were also tested in the same way but no increase of streaming rate could be demonstrated.

With these substances the inhibition was irreversible in high concentrations, causing visible changes in the protoplasm (vacuole formation, coagulation, etc.) while inhibition by lower concentrations was reversible, recovery occurring after an hour or more. The inhibition, then was a toxic one and appeared to be associated with structural changes in the protoplasm itself.

The effect of auxin on streaming

Bonner (1934) reported an increase of respiration in the Avena coleoptile of about 100 per cent due to hetero-auxin extracted from cultures of Rhizopus suinus (3-indol acetic acid), but in a later paper showed that pure crystalline Auxin B had no effect. This was confirmed by Kogl and Haagen Smit (1936) and by Van Hulssen (1936). Bonner stated that the effect of the Rhisppus extrace was due to an impurity which was extracted along with the 3-indol acetic acid. He did not, however, attempt to isolate this impurity and therefore its identity is not known.

Thimann and Marcy (1937) report an increase of streaming by extremely dilute solutions of 3-indol acetic acid (.005-.5mg./liter), and in a later paper (Marcy and Thimann, 1938) suggest this to be the result of increased respiration, without having carried out any respiration

experiments. The increase on streaming reported, however, (from 18 μ / second to 20 μ /second in most of the experiments) is transient, lasting only 5 - 15 minutes. They also report that the presence of fructose in concentrations of 1 to 2 per cent maintains this increase over 30 minutes or more although fructose has no effect alone.

They find the opposite effect with higher concentrations (.1 - 10 mg. / liter) the decrease being transient except in the presence of fructose. This effect they attribute to the acceleration of a second process of respiration which competes for oxygen with the process accelerated by low concentrations. According to this, then, auxin in concentrations up to 10 mg. / liter should cause an increase in respiration, but no such increase has ever been found. (See Chapt. III, p.65)

These experiments were carefully repeated here under the conditions of experiment described in the previous pages and the results with low concentration were found to be negative. Slight effects similar to those described by the above authors were occasionally seen in preliminary experiments, but could not be confirmed later. The 3-indol acetic acid used was prepared and purified (melting point - 167° C) by F.D. Jones and possessed normal auxin activity in producing curvature.

In the case of high concentrations (25-100 mg./liter), however, slight inhibitions were found to occur, but these concentrations fall within a range of toxicity.

The effect of 2-4-dinitrophenol on streaming

The same authors report a decrease in the streaming rate with 2-4-dinitrophenol from 10 mg./liter - 100 mg./liter which returns to normal again as soon as the substance is removed. Since dinitrophenol has been shown to increase respiration in other plant material (Plantefol, 1932) they attribute this effect to an acceleration of a third process of respiration which competes for oxygen with the first two. Their curves showing the effect of this substance, however, suggest a more toxic effect (Fig. 2 A) and the measurements were not continued after removal of the solution in order to determine the presence or absence of an after effect. Moreover, it is to be emphasized that the mechanisms suggested by these authors, even in the case of dinitrophenol, are supported by no measurements of respiration.

Further experiments with dinitrophenol were carried out here and the results are shown in Figure 2 B. It will be seen that the presence of a concentration of 100 mg./liter during 15 minutes causes a rapid decrease in streaming of about 50 per cent, after which recovery does not occur. The inhibition here is so complete that in most cells, even the Brownian movement, characteristic of the protoplasmic particles when streaming is just stopped, also ceases. Yet no coagulation or other characteristics

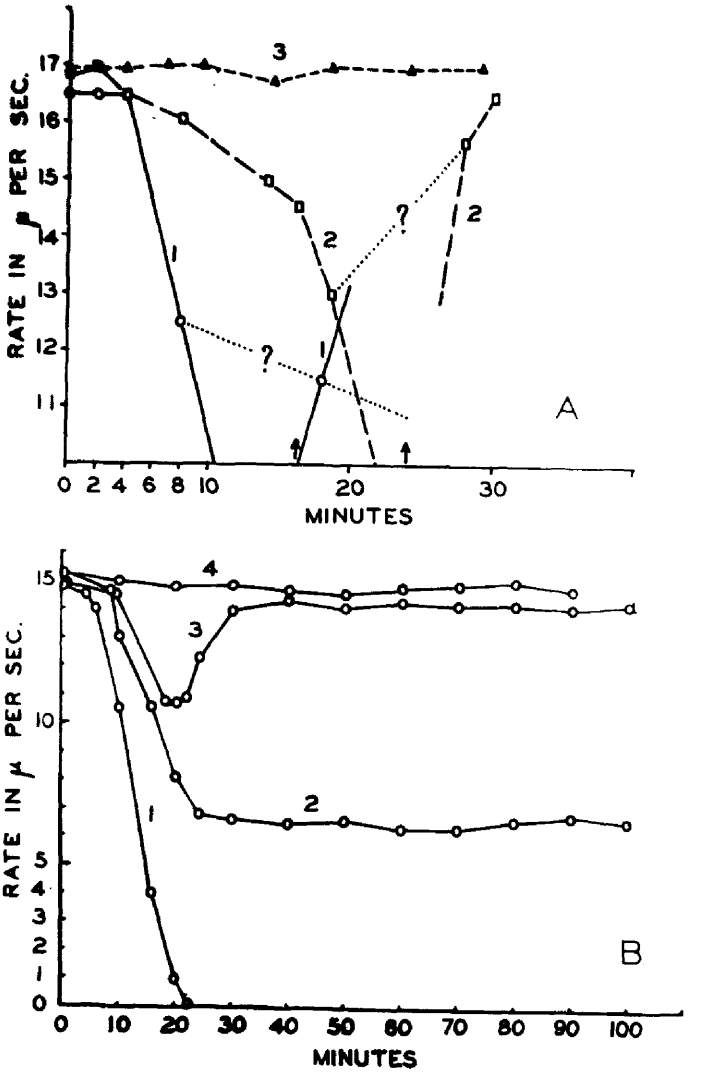

Fig. 8: A. The effect of 2-4-dinitrophenol on the rate of protoplasmic streaming (after Maray Sweeney and Thimann). Solutions removed at the arrows. Curve 1 - 100mg./liter; Curve 2 - 10mg./liter; Curve 3 - 1mg./liter. The dotted lines have been added. B. The effect of dinitrophenol on streaming. Curve 1 - 100mg./liter; Curve 2 - 50mg./liter; Curve 3 - 10mg./liter; Curve 4 - 1mg./liter. Solutions applied during 60 minutes.

of protoplasmic destruction occurs. At 50 mg./liter during 20 minutes the effect is somewhat like that of 100 mg./liter during 15 minutes. The rate falls to about 50 per cent and remains so long after the solution is removed. At 10 mg./liter during 20 minutes recovery occurs at 30 minutes, while at 1 mg./liter no effect appears. Therefore the effect of dinitrophenol appears to be an inhibitory and permanent one in concentrations of 10-100 mg./liter. The dinitrophenol used here was prepared in the laboratory of Dr. N.L. Drake and was later purified by recrystallization three times.

Effect of methylene blue on streaming

In addition to the nitro and dinitro derivatives of phenol, there exists another class of substances which are known to stimulate respiration. This class is made up of certain O/R dyes like methylene blue and pyocyanine, etc. (Harrop and Barron, 1928; Friedheim, 1934; and others.) These reversibly oxidizable-reducible substances act in their reduced forms as auto-oxidizable acceptors of labile hydrogen, and hence cyclically play a role in dehydrogenative cell oxidation. (Wieland, and more recently Engelhardt and Shapott, 1935.) In addition they may sometimes act as direct catalysts of cell oxidation by O_2 (De Meio, Kissin and Barron, 1934). Literature dealing with the effect of these substances on plant respiration, however, is rare. Watanabe (1932) and Ross (1934, 1938) have found acceleration up 66 per cent in Nitella, but similar effects on higher

plants have not been reported. Furthermore no effect of methylene blue on protoplasmic streaming has been reported.

Experiments to determine this effect were therefore carried out. The results, however, were negative. No effect was found in concentrations 10^{-5} to 10^{-2} Molar. Above this range inhibition occurred.

The effect of other substances on streaming

The effect of accessory growth factors such as thiamin, pantothenic acid, ascorbic acid, etc. on plant growth suggests some close relationship with respiration. Of these ascorbic acid has been studied by Clark (1937) with respect to its distribution and effect on growth and curvature in the Avena coleoptile. Clark found no immediate effects of this substance on growth or curvature but found a gradient of reduced ascorbic acid from base to tip and a gradient of oxidized ascorbic acid from tip to base. This O/R activity of ascorbic acid in the coleoptile would appear to be associated with dehydrogenative oxidation. However, no effect of this substance on respiration in Avena has been shown.

Its effect on streaming was determined here. In concentration up to 100 mg./liter no change in the streaming rate was observed.

Marcy (1937) found an increase in the rate of protoplasmic streaming in Nitella caused by ethylene chlorhydrin, and thiourea. This effect, she suggested,

is associated with the effect of these substances in breaking dormancy. (Denny,1928.) In a later paper, however, (Thimann and Marcy, 1937), no effect of ethylene chlorhydrin on the rate of streaming in the Avena coleoptile was found. The remaining substance thiourea, and potassium thiocyanate, an associated substance were used here in order to determine their effect on streaming but they were found to be inhibitory in the used concentrations(100 - 500 mg./liter).

Something must be said here of the effect of saponin on streaming as reported by Clark (1938), although it is not known to be a substance affecting the respiration since it is the only case reported in the literature in which the protoplasmic streaming is supposed to be stopped without a change in transport. Clark, in the course of a study of the relation between electric potentials and auxin transport, reports that saponin stops the streaming yet in no way affects the rate of auxin transport. He does not, however, indicate which saponin is used nor does he indicate properly the concentration of the solutions. Various saponins are available on the market, these varying in composition and nature according to their degree of purification, for example, Merck's "saponin-pure" is a glucoside from Saponaria officinalis and consists of a white, amorphous powder with a pungent odor. It contains traces of sapotoxin but no quillajac acids. Merck's "saponin-purified", however, is an acrid extractive from Saponaria officinalis

and consists chiefly of sapotoxin and lactosins besides salts. It is a yellowish-gray to brown powder and contains quillajac acid. In a like manner there are many other similar differences in other products under the name of saponin. Hence Clark's report is far from specific, the effect on the streaming being likely due to one of any number of substances.[1] His reference to the concentration used in terms of per cent saturation is also far from specific since there exists no well-defined saturated solution of saponin.

Nevertheless an attempt was made here to repeat these experiments concerning the decrease in streaming. Merck's "saponin-purified" was used since it would more likely contain the effective substance in his experiment, and concentrations from 100 mg./liter to 100,000 mg./liter were applied to the coleoptile. In no case was there a decrease in streaming rate during the first four hours of application.

The product commonly known as saponin, then, appears in this case to have no effect on protoplasmic streaming even in very high concentration. It is possible that Clark's "saponin" contains some other component but since this information has not been obtained to date no further work was carried out with these substances. It may be that the high concentration of saponin he used restricted the oxygen uptake of the coleoptile due to its low solubility for oxygen. Further work awaits more

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[1] See Richter, V., The Chem. of the Carbon Compounds, Vol. 2, 1939, pp. 512-525

information from this author.

The effect of KCN on streaming

Since in the previous experiments those substances which have been shown to be accelerators of respiration in other livimg material have no accelerating effect on the rate of protoplasmic streaming in the Avena coleoptile there may arise some doubt as to whether streaming and respiration are closely interrelated. There is the possibility, of course, that the streaming process is dependent upon a respiration system which represents only a small part of total respiration and is different in nature from respiration systems in which the substances in the preceding experiments play a role. This, however, does not seem likely. In order to prove this the effect of KCN on the streaming rate was determined. The work of Warburg and Keilin indicates that KCN pdsons the phaeohemin, or indophenol oxidase in that part of respiration which is catalized by the indophenol oxidase-cytochrome system. Therefore a decrease in streaming by KCN may be attributed to an inhibition of this system, and the remaining respiration dependent upon some other system such as that catalyzed by Warburg's yellow ferment, or flavin enzyme.

The results are shown in Figure 3. It will be seen that only a part of the streaming process is inhibited by KCN even up to the concentration of $\frac{N}{100}$. At higher concentrations the poison ceases to be specific and other systems are affected. In all cases the inhibition

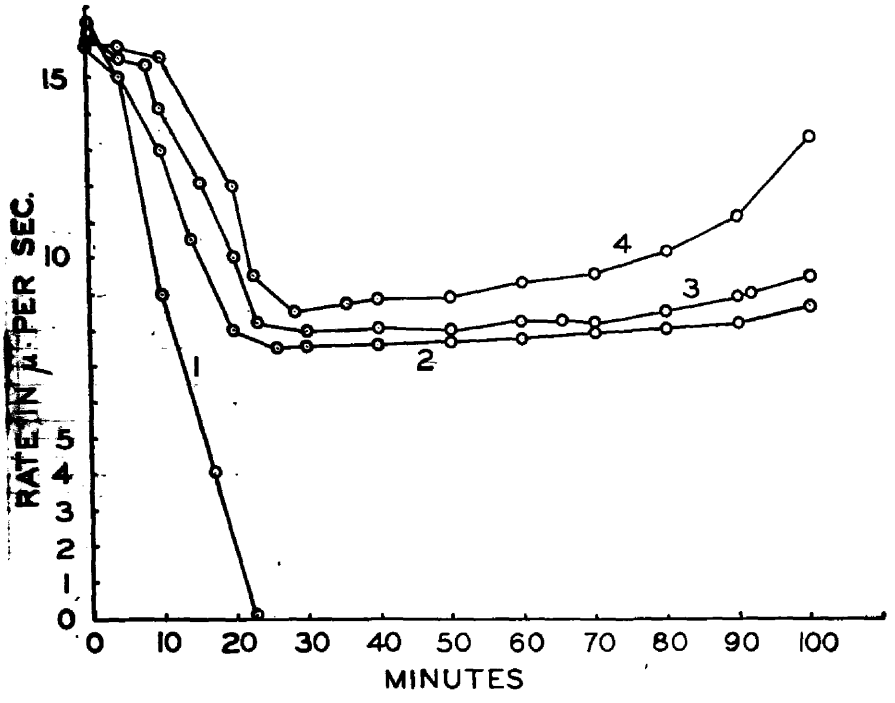

Fig. 3: The effect of KCN on the streaming rate. Solutions applied over 25 minutes. Curve 1 - $\frac{N}{10}$; Curve 2 - $\frac{N}{100}$; Curve 3 - $\frac{N}{3000}$; Curve 4 - $\frac{N}{4000}$

was reversible, recovery occurring after an 25 minutes or more, except, of course, in the case of long exposure to high concentrations where apparently some secondary inhibition occurred.

DISCUSSION

The effect of ether, although it does not fulfill the purpose for which it was used, coincides favorably with the effect of such anaesthesia on auxin transport in the Avena coleoptile. Van der Wey (1932, 1934) found that an ether atmosphere inhibited auxin transport in the coleoptile when introduced in concentration of 10 - 40%. At 40% transport was so completely inhibited that its rate could be accounted for by diffusion alone. Furthermore, in no case did he find an increase of transport at low concentration. It appears, then, that effect of ether on transport acts through a decrease, and finally cessation, in high concentration, of the protoplasmic streaming rate, thus limiting intracellular translocation of auxin. Since the ether was applied by Van der Wey in the gaseous state its concentration effect cannot be directly compared with that on streaming here, where the substance was applied in solution. However, simple calculation shows the effect to be of the same order.

The negative effect of auxin regarding streaming acceleration agrees with unpublished results of Bottelier, who during the course of his earlier work on streaming

attempted to demonstrate an increase there with pure auxin B, purified by Dr. Kögl. The effect obtained by Thimann and Marcy might have been, as in the case of the respiration increase obtained by Bonner, due to an impurity, since the auxin they used was prepared four years ago and, without doubt, from Rhizopus culture. Moreover, Thimann and Marcy allowed only five minutes for recovery of the coleoptile [illegible] cutting as compared with one hour in this and Bottelier's investigation. It is possible that the auxin in their work played a role in some manner during the wound effect.

Their theory that auxin increases respiration does not fit the results of Bonner (1936), Van Hulssen, (1936) or Kögl (1935), [illegible] Smit, for even though they describe the effect as transient, auxin should in higher concentrations (1-10 mg./liter) by increasing both the postulated streaming-accelerating process and the streaming-inhibiting process, cause a measurable increase in oxygen consumption. Moreover, this conception is not favored by the results obtained here with KCN. If auxin were to stimulate a single process apart from the total respiration it would not be expected that this same process would be only partially inhibited by KCN, for such partial inhibition shows streaming to be dependent upon two or more processes. Moreover, the chemical properties of the auxins do not make their function as respiration enzymes seem plausible. Without further data on respiration such a theory is not justified.

The negative effect of methylene blue in a KCN

sensitive system such as that concerned with streaming is not an exception. According to Barron and Hoffman (1930) the rate of dehydrogenative activity of these O/R substances must exceed that of the normal respiring material in order for any favorable effect to occur. Moreover, they can only enhance respiration in those cases where the normal rate is insufficient to eliminate aerobic glycolysis.

The lack of acceleration with ascorbic acid may be explained in the same manner since this substance acts by virtue of its oxidation reduction properties.

The stimulatory effect of 2-4-dinitrophenol on respiration is not a universal phenomenon. Indeed, Plantefol (1932) has shown it to be effective in some plant tissue but no such increase has been demonstrated for the Avena coleoptile. Moreover, Genevois and Saric (1932) found that not only was there no acceleration in yeast but that a concentration of 100 mg./liter caused a 90% inhibition. The nature of the inhibition in the Avena coleoptile appears to be due to some effect on the protoplasm. Protein denaturization effect of similar phenolic substances such as picric acid (2-4-6-trinitrophenol), etc. is well-known to the histologist. Therefore, it is possible that a similar affect may occur here, comparable to partial histological fixation in which the protoplasmic particles become held by partially denaturized protoplasm with a minimum of visible coagulation.

The occurrence of a cyanide sensitive system contributing to a part of the streaming process necessitates,

obviously, further investigation of the effect of various substances on the respiration of the coleoptile. This is taken up in the following chapter.

CHAPTER III

FACTORS INFLUENCING RESPIRATION

METHOD

Principle

Since the Avena coleoptile is an ephemeral organ with a relatively low respiration rate attempts to study this process have been limited by the precision of the methods used in its determination. The manometric method, infallible with most other material, necessitates the use of many coleoptile sections and very small vessels. Bonner (1934) was the first to use this method with coleoptile sections and found it necessary to use a technique employing small conical vessels without an alkali well. The O_2 uptake was measured by the decrease in pressure due to respiration and depended upon the different solubilities of CO_2 and O_2 in the liquid present. It was calculated by a method given by Crafton (1929) and was based on the assumption that the respiratory quotient equals 1. The values obtained were only relative, therefore, and the Q_{O_2} (cm.^{3}/hour per mm. coleoptile length) was found to be of the order of 0.15. Even with this special adaptation of the Warburg Manometric method, Bonner found it necessary to place in each vessel from 89 to 150 coleoptile sections of 3mm. length, and the extensive wound surface caused extreme variability in oxygen uptake during the first four hours or more.

In a later paper (1936) Bonner used standard vessels with alkali wells in the Warburg method but the vessels were apparently very small, for he was able to use only twenty 3 mm. coleoptile sections in each vessel. The Q_{O_2} thus determined was higher than in the preceding method and was of the order of 0.50. Kögl and Haagen Smit (1936) and Van Hulssen (1936) using about the same method found a similar value.

There is a distinct disadvantage, however, in using such a large amount of material in each cell. Individual morphological differences and age differences are bound to occur in each coleoptile section and these, even though slight, may well mask the effect of an external factor studied in the experiment through differences in reaction capacity. It would be far better, if possible, to measure the O_2 uptake of a single coleoptile. This of course cannot be done with the Warburg apparatus.

The Fenn differential volumeter respirometer offers a method for measuring extremely low respiration rates over short intervals but has the disadvantage of necessitating a very high precision of temperature control. Thimann (unpublished) has designed a highly sensitive respirometer based on the Fenn principle and operated in a constant temperature bath within a constant temperature bath. Yet, many variations still occur when only one coleoptile is used. These variations are apparently due to inherent faults in the method, for example, the lag between the liberation of CO_2 by the coleoptile and the

absorption of CO_2 by the alkali solution, leakage at ground glass joints, as well as slight volume changes caused by small fluctuations of temperature. All these factors are so high with respect to rate of volume decrease due to O_2 uptake that they introduce serious difficulty in obtaining reproducible results.

It appears, then, that the rate of oxygen uptake of a single coleoptile section is below the lower limit of measureability with existing manometric and volumteric methods. If it is possible to follow such small changes in oxygen uptake a method based on some other principle must be used. Such a method is offered by the principle of the Heyrovsky polarograph.

This principle is based on the interpretation of current-voltage curves obtained from electrolysis of solutions of electro-reducible or electro-oxidizable substances in a cell in which one electrode consists of mercury dropping in very small, slow-forming drops from a narrow glass capillary. From these voltage-current curves resulting from oxidation or reduction at this dropping mercury electrode both the concentration and the species of substances in solution can be determined. Quantitatively the method is best suited for determination of concentrations as low as from 10^{-5} to 10^{-2} molar.

In practice the electrode cell is connected, in series with a galvanometer, to a battery and potentiometer such that the applied E.M.F. is known or can be computed.

The current-voltage curves are then obtained by gradually increasing the applied E.M.F. and noting the current measured by the galvanometer. These curves may be obtained by manually increasing the applied E.M.F. and plotting the measured currents against applied voltages or they may be obtained automatically with the instrument known as the polarograph. This device, devised by Heyrovsky, provides a means of automatically recording the curves on a rotating drum in a fraction of the time required to obtain them manually. It is of much value in theoretical work but is not essential in routine analysis.

As the voltage is increased the current increases slowly until the decomposition potential of the reducible substance in the solution is reached. This part of the curve is known as the residual current and is more or less flat. Above the decomposition potential the current increases sharply until at a region determined by the concentration of reducible substance it ceases to rise. This flatter part of the curve is known as the limiting current or diffusion current. The diffusion current remains constant with increasing voltage (in the ideal curve) until the decomposition potential of some other reducible substance in solution is reached. The current flowing depends upon the rate of diffusion of the reducible substance to the electrode and this in turn depends upon the concentration.

Since a fresh surface of mercury is constantly being exposed through the increase in the drop size the

dropping mercury electrode gives reproducible results. In cases of electro reduction it is used as the cathode, the anode being formed by the mercury which collects at the bottom of the cell.

This method has been used for nearly sixteen years in chemical analysis of various solutions and during this time numerous difficulties have been overcome such that the method today has much practical advantage and many applications in the analysis for a great many different substances. One of these substances can be oxygen in solution since it is found to be reducible at the dropping mercury electrode. Vitek (1933) first made use of this in the determination of O_2 in various technical applications.

Use of the method for meas uring respiration, however, has not been introduced until recently. Petering and Daniels (1938) have recently shown the remarkable adaptability of the method in measuring the photosynthesis of Chlorella and the respiration of erythrocytes and various animal tissues. The dropping mercury electrode is used to measure the concentration of oxygen in a closed cell containing the material in nutrient solution at intervals during the course of respiration, and from this the O_2 uptake can readily be determined.

In the case of low respiring material such as erythrocytes from dog's blood in their experiments, these authors find that the O_2 uptake can be followed over five minute intervals with a remarkable degree of accuracy,

whereas the O_2 uptake of some material could not be followed in the Warburg respirometer. In their apparatus the smallest measurable change of O_2 concentration was of the order of 0.112 $mm.^3$ or $1.6X10^{-7}$ g. ($5X10^{-9}$ mole). Moreover, the values obtained for more rapidly respiring material checked with the Warburg analysis and suffered no decrease from the toxic effect of mercury.

Another contribution is offered by these same authors in that they dispense with the need of following complete current-voltage curves for O_2 determination. Instead, the current is measured at a predetermined voltage just below the decomposition potential of oxygen, and again at a second voltage just above the decomposition potential in the flatter region of the curve associated with the diffusion current, just below the decomposition voltage of the next reaction that occurs. The difference between the galvanometer deflections at these two voltages is found to be proportional to the concentration of oxygen in solution. The two voltages were found to be 0.1 and 1.0 volt.

Making use of an increment method such as this, one does not need the costly recording apparatus devised by Heyrovsky and yet one need not follow the tedious procedure of following current-voltage curves manually. Moreover, the concentration of oxygen in solution may be determined within several seconds, a distinct advantage in following a rapid change in concentration.

A further advantage of the dropping mercury electrode

as its low sensitivity to temperature change. According to Kolthoff and Lingane (1939) the diffusion current increases only about 1.6 per cent per degree rise in temperature. Therefore it is necessary from the analytical point of view to control the temperature only to $\pm 0.5^{\circ}$ C in order to keep this error within 1 per cent.

There are a number of other factors which by coincidence make this method ideal for respiration measurements. These are as follows:

(1) The carbon dioxide given off by the respiring tissue has no effect on the measurements, since the CO_3 ion is not reducible or oxidizable at the electrode. Therefore no specific CO_2 absorbent need be added to the cell. Moreover, the increase in H^+ ion concentration due to liberation of CO_2 also has no effect on the diffusion current measured.

(2) Even with frequent measurements there results no measureable degree of local change in oxygen concentration through electrolysis. This can be shown by simple calculation.

(3) The solution itself is in no way altered by the electrolysis since the degree of decomposition at the cathode is too low to be measureable even after repeated measurements.

(4) The apparatus is simple, inexpensive and requires no special or detailed calculations for the determination of oxygen and once calibrated the O_2 uptake can be read

directly off the galvanometer scale.

(5) The measurements are carried out in solution. This is of advantage in that there is no need of absolutely gas tight ground glass joints since the diffusion of oxygen through water is too slow to be effective here. Furthermore, any number of solutions can be added during the course of an experiment without the use of side wells by simply replacing the solution electrolyzed, since the volume is not a factor in measurement.

(6) The electrolyzing current is far too low (maximum of $50X10^{-5}$ amperes) to have any detrimental effect on the tissue. Since the resistance of a coleoptile section is of the order of 100,000 ohms/cm (du Buy and Olson, 1938) and that of the solution is many times lower, the current passing through the plant is far below the threshhold for a current which would affect metabolism.

There are, of course, some limitations and disadvantages to the method but these will be taken up later.

No investigation utilizing the method for measuring respiration of plant tissue has appeared in the literature even at the time of writing. Therefore, the experimental work done here dealing with the adoption of the method is included somewhat in detail in the following pages.

Apparatus

The apparatus used is similar to that used by Petering and Daniels but with several improvements. The cell

itself (Fig. 4) of Pyrex glass is much smaller, measuring about 0.6 cm. outside diameter and about 4 cm. in height, its working volume being a little over 1cc. This size was found after trial and error to be of optimum size for these measurements since a maximum sensitivity is obtained with enough volume to allow for unrestricted oxygen uptake of a single coleoptile over a sufficiently long period without need of refreshing the solution. A side arm is provided at the lower part of the cell, leading to a small reservoir which serves as an overflow vessel for the mercury. This arm is made such that the anode mercury in the vessel remains at a constant height, restricting the cell volume to about 1 c.c.. A constant cell volume is thus provided, yet the cell may be immersed in a bath without contamination of the anode mercury. Since the flow of mercury is very slow it is necessary to withdraw the mercury from the overflow vessel only once every two hours. The anode connection is provided by a fused-in platinum wire leading to a side arm containing mercury into which is dipped the anode lead to the galvanometer.

Since an all glass system is desirable the dropping mercury capillary is led directly into the mouth of the cell, which is flared to fit the taper of the capillary. (A ground glass joint was first used here but was found to be unnecessary.) This allows for convenience in removing the capillary to add solutions, etc.

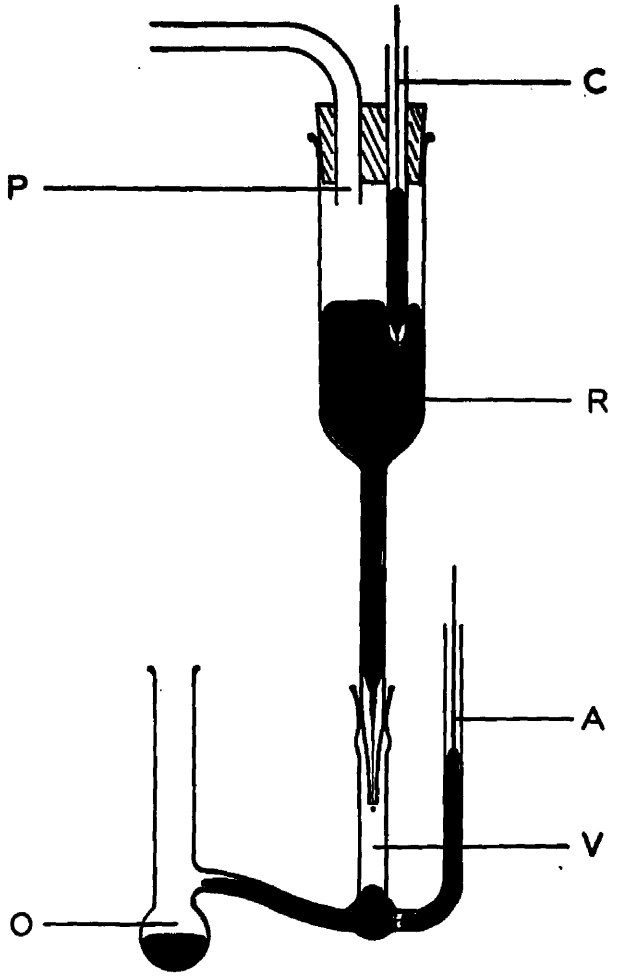

Fig. 4: Diagram of electrode assembly. A. Anode connection; C. Cathode connection; O. Overflow vessel; V. Electrode vessel; R. Mercury Reservoir; P. Pressure inlet.

Heyrovsky and his school, and Petering and Daniels as well, made use of gravity in maintaining the flow of mercury, the pressure being adjusted by raising or lowering the mercury reservoir which was connected to the capillary by a rubber tube. This, however, is undesirable. It is nearly impossible to remove all small particles (bloom) from inside the rubber tube and these are likely to be carried down to the capillary to clog its small opening. Moreover, even pure gum rubber contains enough sulphur to react with the mercury, forming a scum of mercuric sulphide which may by contamination affect the electrode phenomena, and may even clog the capillary as well. Therefore an all glass system similar to that used by Müller, Garman, Droz, and Petras (1938) was used in which the capillary was fused directly to the mercury reservoir. The flow of mercury was provided by a constant pressure on the surface of the mercury in the reservoir maintained by a system such as is shown in Figure 5. The pressure is provided by the rubber bulb and measured roughly with the manometer. It is then adjusted carefully to maintain the required drop rate by opening the micro-bubble release. The cathode connection is provided in the usual manner by a platinum wire dipping into the mercury in the reservoir. Since the pressure used to provide for the flow of mercury was rather high the rubber stopper through which this connection was passed was held tightly by the clamp arrangement shown in Figure 25.

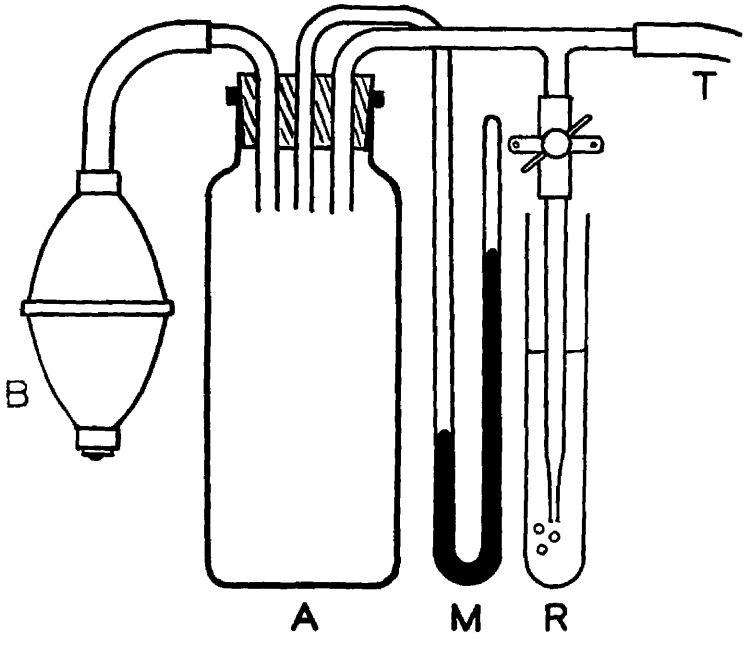

Fig. 5: Arrangements for maintaining pressure on the mercury. B. Rubber bulb; A. Air chamber; M. Manometer; R. Pressure regulator; T. Pressure lead to mercury reservoir.

This system was found to be far more convenient and flexible than the usual levelling system and almost no trouble due to clogging of the capillary tube was experienced.

The capillaries were hand drawn from 1 mm. Pyrex capillary tubing, the walls of the tubing being heated in the flame for some time before drawing out in order to insure a tip with a small bore and a thick wall. They were selected for the proper lumen and cut off squarely using a small, rough-sharpened, hard steel razor blade. Selection was then made by trial and error for optimum drop rate (about 1 drop/second), drop size, and maximum galvanometer deflection.

The circuit is a simple one consisting in the main of a Leeds and Northrup student potentiometer adjusted to provide the proper applied voltages, and the electrode cell in series with a Leeds and Northrup Type R galvanometer. (Sensitivity 0.49 μ v/mm., coil resistance 6.7 ohms, period 4.8 seconds, critically damped by a shunt of 52 ohms.) The latter was made free of vibration by mounting on a six inch iron pipe from the concrete floor. The suspension was turned so that readings could be taken over more than half the length of the scale, which was placed at an optical distance of 30 inches from the reflecting mirror. The working current of the potentiometer was balanced against a standard cell in the usual manner.

The mercury used for the cathode was not vacuum

distilled but was carefully cleaned several times by passing through glass cloth into first a column of 10% KOH, then through acidified $Hg(NO_3)_2$ and finally double distilled (glass in glass) water. This procedure was found to provide adequate purification.

Choice of ground solution

In the usual polarographic method electrolysis is carried out in a suitable "ground solution" or environment solution which favors the magnitude of the diffusion current and the detection of it from the rest of the curve. Many such ground solutions are given in the literature and several are studied in detail in Hohn's (1937) treatise on polarographic analysis. However, in this investigation a solution must be provided which not only favors an ideal current-voltage curve (polarogram) but, moreover, favors the normal metabolism of the plant. It must provide a non-toxic medium of low osmotic value with its components in a suitable ratio to allow for antagonism of toxic ions such as those which might occur from mercury. It must also be of suitable composition such that certain salts present in the plant which might affect or mask the O_2 polarogram will not diffuse out into the solution. Obviously such a solution can only be attained by trial, using those which appear likely to fit these criteria.

In determining the effect of these ground solutions both on the coleoptile and on the polarogram, complete current-voltage curves were followed manually, first

with the solution alone and later with the solution plus the coleoptile after it had remained in the solution for some time. If in the first instance the curve followed the same general shape as that given in the literature for oxygen it was assumed that the solution was favorable for the oxygen curve. If in the second instance the characteristics of the curve differed none from the characteristics of the first curve (other than decrease in height due to O_2 uptake), it was concluded that the solution was favorable to the coleoptile in the respect that no marked diffusion of ions out of the coleoptile occurred.

Something must be said here of the maxima which arise at the decomposition potential. These are common to all polarographic analysis and arise through the electrostatic adsorption of reducible substances on the mercury thus allowing a current increase beyond that of the diffusion current. They are reproducible but not proportional to the concentration of reducible material in solution and therefore must be suppressed. This is accomplished by adding some other adsorptive material such as gelatine, soaps, dyes, etc. which is adsorbed on the mercury in the place of the reducible substance.

In these experiments it was found that enough colloidal material to suppress the maxima is provided by the cut cells of the coleoptile even after rinsing the material several times before the determination. Moreover, in the case of oxygen the maxima do not present a serious

difficulty when the increment method is used. The ideal O_2 current-voltage curve has three steps or flat portions between zero and one volt, and since the maximum appears over the second of these flat portions (0.4 v) it lies between the two voltages used in the measurement (0.1 v and 1.0 v). It is therefore independent of the deflection measured. If an excess of colloidal material is present, however, a serious error can result through the suppression of the whole curve. In this investigation such was prevented by rinsing, allowing the coleoptile section to remain in the solution for some time before the experiment during which the excess colloidal material could diffuse out into the solutions. In no cases were the maxima entirely suppressed during the determinations.

Ringer's solution has been useful in animal work in providing a suitable medium for the isolation of tissue for experimental purposes. Since it provides a simple non-toxic, yet suitable environment for living cells it was tried here more or less as a preliminary experiment. It was found to be quite favorable, with the exception of a slight maximum in the region just above the diffusion current. (Fig. 6). Moreover, Ringer's solution is not ideal for plant growth as plant nutrition studies bear out. Therefore several plant nutrient solutions were tried with some success. The effect of one of these, Shivés 3 salt solution (N_o. R5C2) is shown in Figure 7.

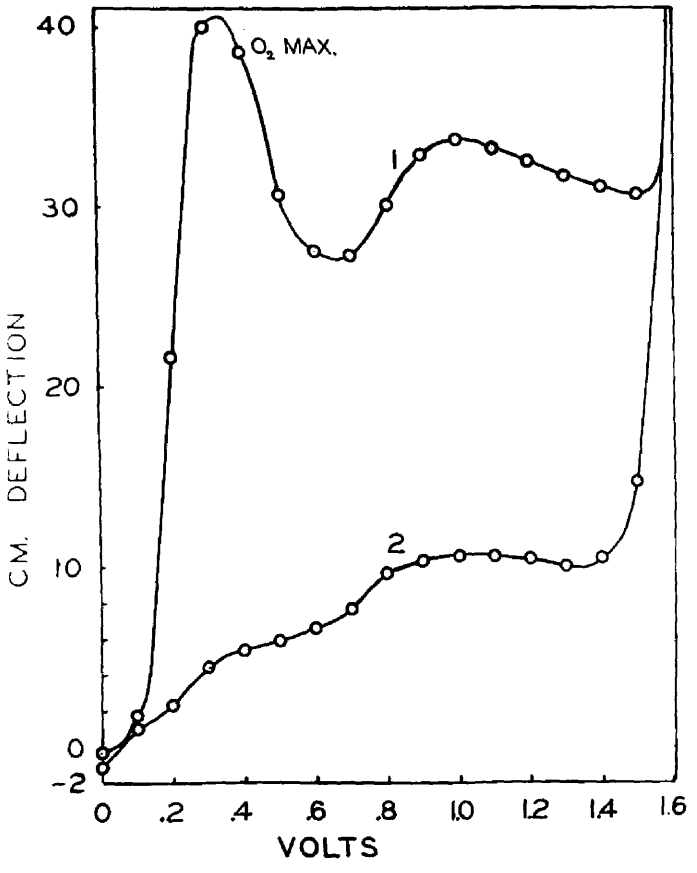

Fig. 6: Current voltage curve of Ringer's solution. Curve 1 - Solution alone; Curve 2 - solution + coleoptile after several hours in electrode vessel.

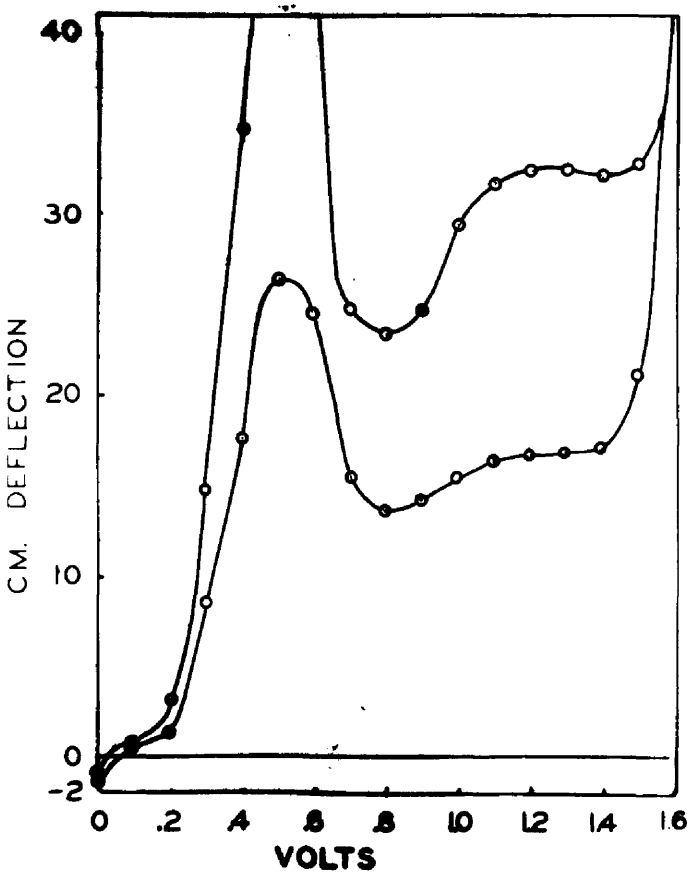

Fig. 7: Current voltage curve of Shive's solution. Upper curve - solution alone; lower curve - solution + coleoptile

This solution also gave favorable results but the same maximum occurred in the region of the diffusion current. The cause of this maximum and that shown by Ringer's solution is not known. Since it is not typical of the oxygen curve, further solutions were tried. The most favorable was found to be a modified Knop's solution of the following composition: $Ca(NO_3)_2 \cdot 4\ H_2O$ - 2.5g.; KNO_3 - .8g.; KCl - .6g.; $MgSO_4 \cdot 7\ H_2O$ - .35g.; KH_2PO_4 - .15g.; redistilled H_2O - 1000c.c. This stock solution is diluted to 1 part:5 parts of redistilled H_2O. Its curve is shown in Figure 8.

Since the curve is characteristic of the oxygen wave and shows no change after the course of respiration the next step is to prove that with its use no toxic effect of the mercury in the cell occurs. The criteria used for toxicity were changes in the rate of protoplasmic streaming and in the rate of elongation of a coleoptile placed in the cell during frequent electrolysis over a period of about six hours. No change in the rate of streaming or the rate of elongation occurred. All solutions applied to the coleoptile in the following experiments were made up in a nutrient solution of the above concentration.

Calibration

For each capillary used in the experiment calibration was carried out for several concentrations by measuring deflection against known oxygen concentration.

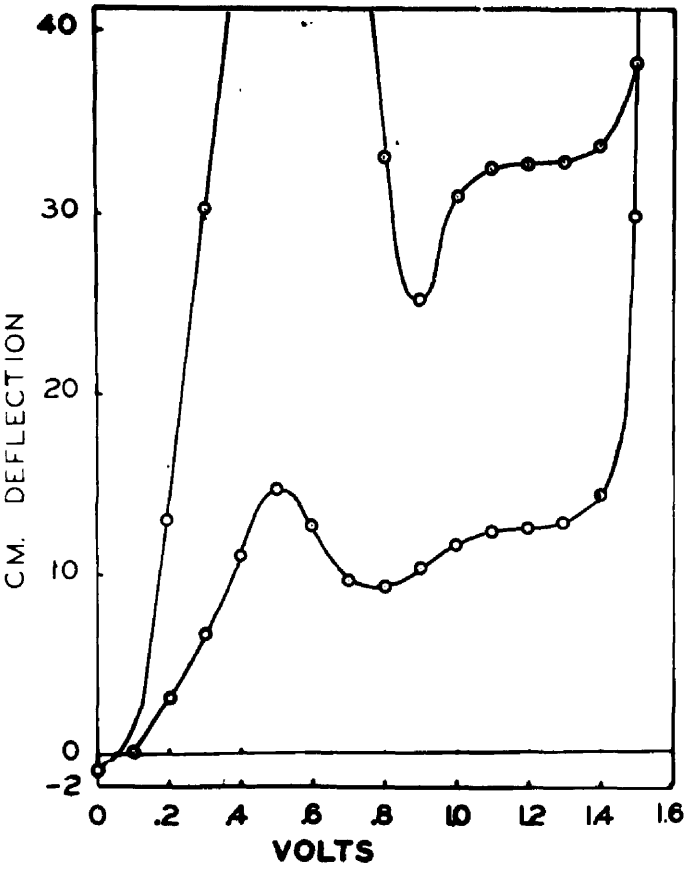

Fig. 8: Current voltage curve of nutrient solution used throughout the experiments. Upper curve - solution alone; lower curve - solution ⊣⊢ coleoptile

This was accomplished by saturating a large quantity of nutrient solution with hydrogen and measuring the deflection of several samples withdrawn during the course of saturation. These same samples were then analyzed for oxygen in solution by the Winkler method. Extreme care was used in withdrawing samples to insure that no air could dissolve in them, the oxygen analysis being carried out as soon as possible. The Winkler method used was that as given by Theroux, Eldridge, and Mallman (1936), with the exception that samples were collected in 50 c.c. glass stoppered, volumetric flasks. After addition of the reagents two 25 c.c. aliquots were titrated with 10 c.c. micro-burrettes using $\frac{N}{200}$ $Na_2S_2O_4$. The results of such a calibration are given in Figure 9 and it will be seen that there is a straight line relationship between O_2 concentration and deflection over the range of concentration in which determinations are made. (2-9 mg./liter)

Procedure

In the following experiments the coleoptile was removed from the plant and the tip (upper 2 mm.) removed. The space in the center of the coleoptile cylinder was then filled with nutrient solution and a small, short, glass rod inserted in the lower end to serve as a weight to keep the coleoptile in position during the measurements. It was then infiltrated and allowed to remain in the nutrient

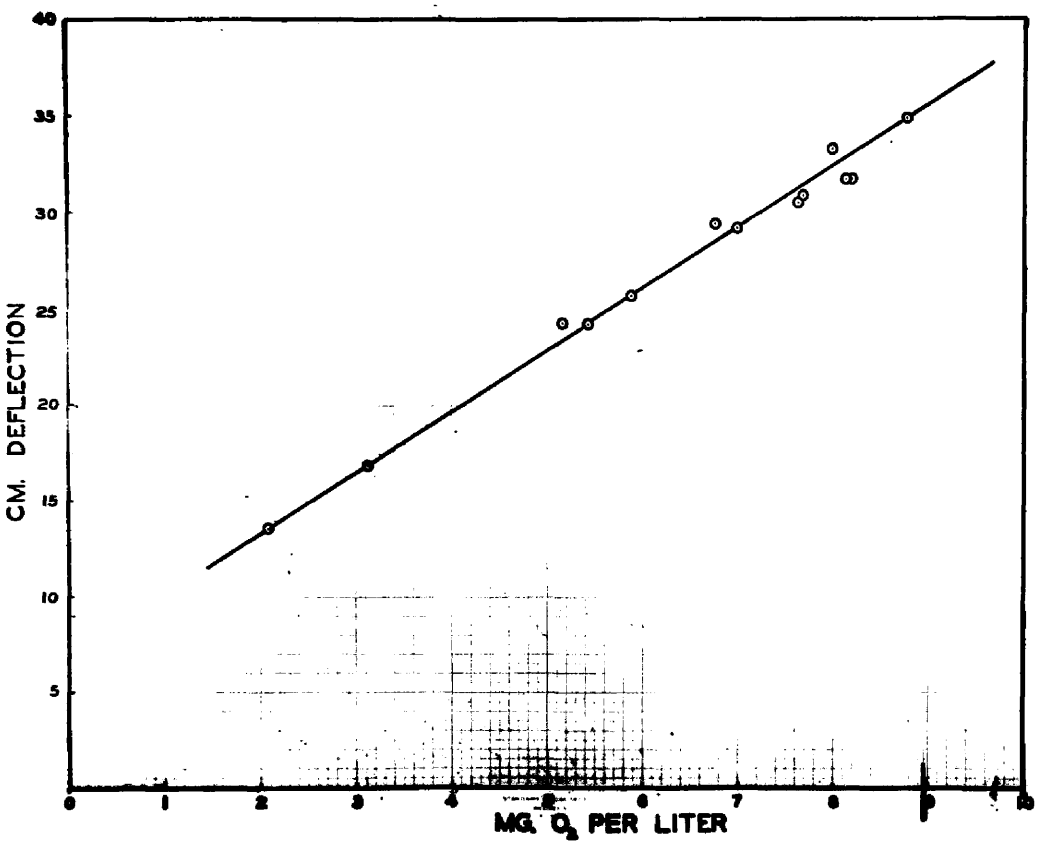

Fig. 9: Calibration curve (drop rate of capillary 1.4 drops/second)

solution for an hour or more during its recovery from the wound shock of cutting. It was then carefully placed in the electrode cell, the solution added, and the dropping mercury electrode lowered into the cell.

During the measurements the galvanometer oscillates slowly due to the dropping mercury at the cathode. The maximum "throw" of this oscillation, since it is remarkable reproducible, was used in measuring deflection in all the experiments.

Experimental results

It was found after several determinations that the method was more than adequate for measurements of the respiration of a single coleoptile section and that, when the system was in perfect order (contacts clean, mercury clean, etc.), measurements could be made over intervals as short as 2 minutes without much variation. The curves follow a steady decrease in oxygen tension corresponding to 1-2 cm. galvanometer deflection over a 5 minute interval. According to calibration, 1 cm. deflection corresponds to about 0.25 mg./liter or 0.145 c.c./liter change in oxygen concentration. Since the electrode cell is about 1.2 c.c. in volume this change is caused by an uptake of only 1.3 $mm.^3$ of oxygen by the plant. The scale can be read accurately to 0.5 mm. corresponding to an oxygen uptake of only 0.065 $mm.^3$, but from measurements taken on a solution alone when the cell is immersed in a bath at $23^{\circ} \pm .01$ there was found to be

variation of ±1 mm. deflection, leading to an error of ±0.13 $mm.^3$. Further variation was sometimes encountered when contacts or the capillarywere not clean.

One of the first interesting phenomena encountered was the marked change in respiration rate below a certain oxygen tension. In Figure 10 the slope or respiration rate is seen to be a straight line[12] until a certain low oxygen tension is reached. At this point a new and lower rate occurs which is about 50 per cent of the normal rate. This point, the "critical oxygen tension", was found to be more or less constant for the material used at first, but when younger or older coleoptiles were used it was found to vary accordingly, being higher (about 0.45 vol.% O_2) for young coleoptiles and lower (about 0.25 vol.% O_2) for old coleoptiles. From determinations of the rates of coleoptiles of various ages this relationship between age and the critical oxygen tension was found to be consistent almost to the extent that the age of the material could be judged by the critical oxygen tension. In Figure 11 are shown the oxygen consumption curves of several coleoptiles of different ages with their critical oxygen tensions. It will also be seen from these curves that the rate of respiration or Q_{O_2} is higher for younger coleoptiles than for older coleoptiles as was also found by Bonner (1934), being

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12 The gradual curve at the beginning is caused by air in the coleoptile cylinder from which O_2 is withdrawn instead of from the solution. Care was taken to prevent this in subsequent experiments.

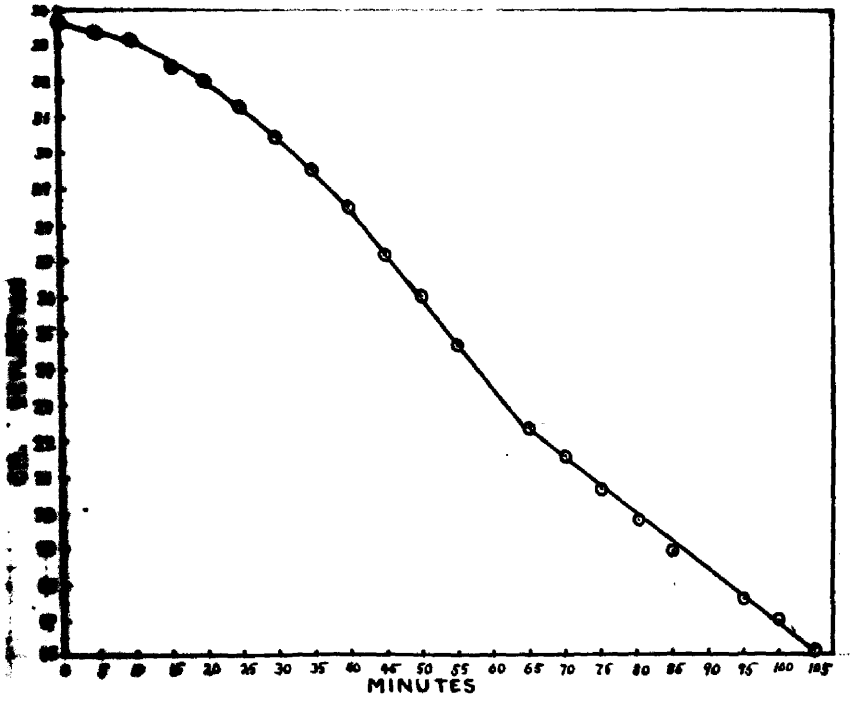


Fig. 10: Oxygen consumption curve showing break at the critical oxygen tension

about .80 for 3 cm. coleoptiles and about .90 for 5 cm. coleoptiles. The ratio of the normal respiration to that below the critical O_2 tension, however, is seen to be the same (about 2:1).

That this critical oxygen tension was not a result of pH change of the solution due to CO_2 liberation, or of the effect on the electrolysis by some substance contributed by the coleoptile, could be shown by merely withdrawing the solution and allowing it to slowly drop back into the cell again. With the solution thus aerated the critical oxygen tension was found to be reproducible as many times as this procedure was repeated. Also if the solution is replaced by a new solution the same critical O_2 tension is found.

This limiting effect at low oxygen concentration is, then, a characteristic of the coleoptile itself and two possible explanations can be given for it. First, it could be the result of a decrease or stop in respiration of one part of the coleoptile, the respiration of the remaining part contributing to the new slope. Second, it could be the result of a decrease or stop in one system of respiration (cytochrome-indophenol oxidase system) the remaining system (flavin enzyme system) contributing to the second slope. In order to determine which of these possibilities was the factor involved, the following experiments were carried out.

The respiration of a coleoptile was followed over a period of 70 minutes and its curve plotted as in Figure 11. The coleoptile was then removed from the cell and cut lengthwise. After about an hour in nutrient solution for recovery this same coleoptile was placed in the cell again and its respiration followed over the same period. The two curves are shown in Figure 12 and will be seen to be similar, the critical oxygen tension being the same for both. This was repeated with coleoptiles of other ages with the same result.

The sudden decrease in respiration rate at the critical oxygen tension cannot, then, be due to a restriction of oxygen to those cells inside the coleoptile cylinder since it still occurs when the cylinder is opened by longitudinal cutting. It therefore seemed possible that the cells near the tip of the coleoptile could stop respiration at low oxygen tension since their surface is small with respect to the protoplasm within. This is not likely, however, since the change in cell length and hence cell surface from tip to base in the coleoptile is gradual whereas the limiting effect of oxygen on respiration/3 is a sharp change. The rates of respiration of tips were compared with those of bases, however, and were found to be the same. Moreover, the same critical O_2 tension was common to both.

It seems more likely, then, that the second

/3 If determinations are carried out over several hours, the limiting oxygen tension for all respiration is reached. Since this point falls in the region of O_2 concentration where calibration is beyond the sensitivity of the Winkler method, the curve was not followed this far.

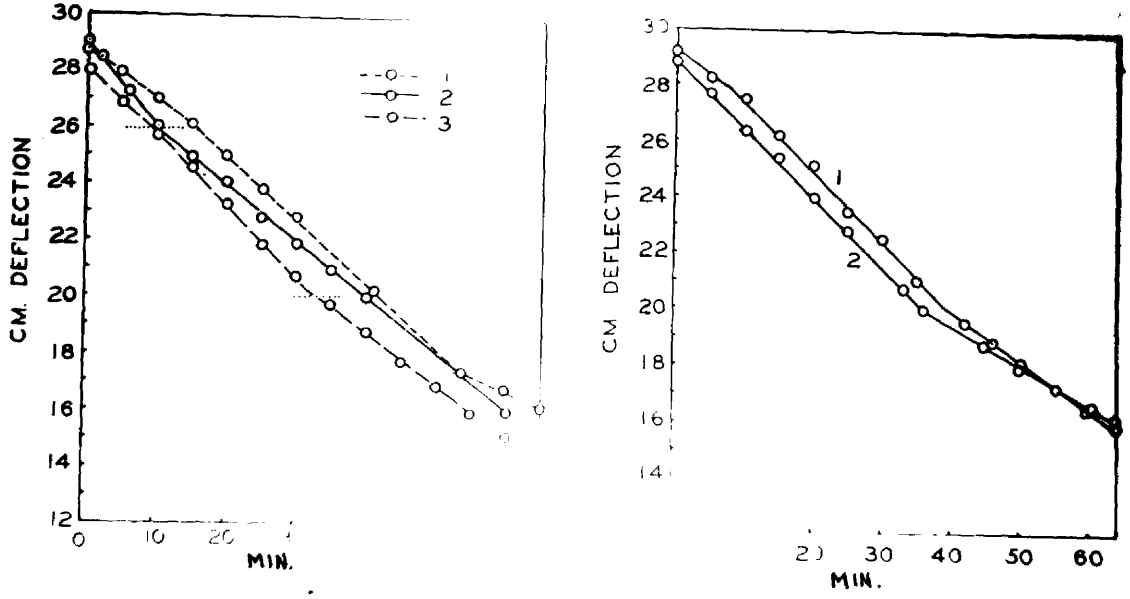


Fig. 11: Oxygen consumption curve of coleoptiles different ages showing differences in critical oxygen tension. Curve 1: 130 hour coleoptile, Curve 2: 80 hour coleoptile, Curve 3: 95 hour coleoptile.

Fig. 12: Showing the effect of cutting the coleoptile cylinder lengthwise. Curve 1: Before cutting, Curve 2: After cutting.

possibility named previously is the factor involved. Therefore it seemed appropriate to investigate the effect of KCN on the respiration in order to determine whether or not a cyanide-sensitive respiration system existed as in the case of the streaming process as found in Chapter II. It was found, however, that KCN even in low concentration causes a shift in the polarogram as shown in Figure 13. Hence, if the KCN were placed in the cell during the respiration measurement it would be necessary to recalibrate the instrument for each concentration of KCN used. This recalibration would also be necessary, of course, for any other substance influencing the polarogram in each concentration added to the cell. Since this would involve a lengthy procedure in all experiments dealing with the effect of such substances on respiration it was decided to treat the coleoptile with each substance and then, after careful rinsing, place it in the electrode cell. Using this procedure it was found that no effect of KCN on the polarographic curve resulted. The coleoptile sections were treated with KCN for 25 minutes, followed by a 5 minute rinsing period.

In Figures 14, 15, and 16 are given some of the results of the KCN experiments. It was found that $\frac{N}{5000}$ KCN reduced the respiration rate about 50 per cent while concentrations up to $\frac{N}{100}$ KCN reduced it to the same degree. This agrees with the effect of KCN on streaming, which was found to decrease, but not inhibit the streaming. Moreover, it will be seen from the figures that the reduced rate of

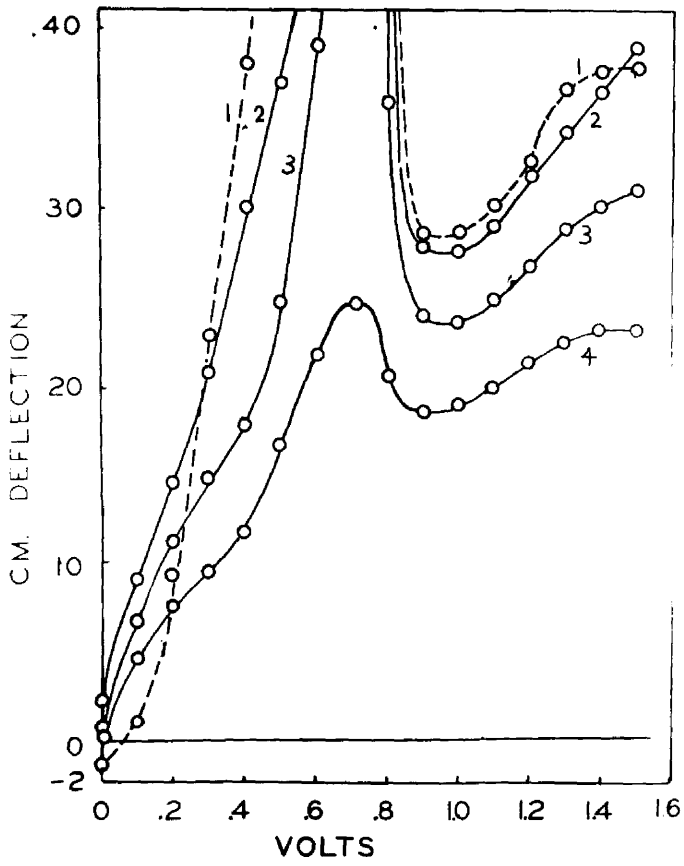


Fig. 13: Current voltage curve of nutrient solution + KCN showing the shift caused by KCN. 1. Normal curve. 2. $\frac{N}{10}$ 3. $\frac{N}{100}$ 4. $\frac{N}{1000}$

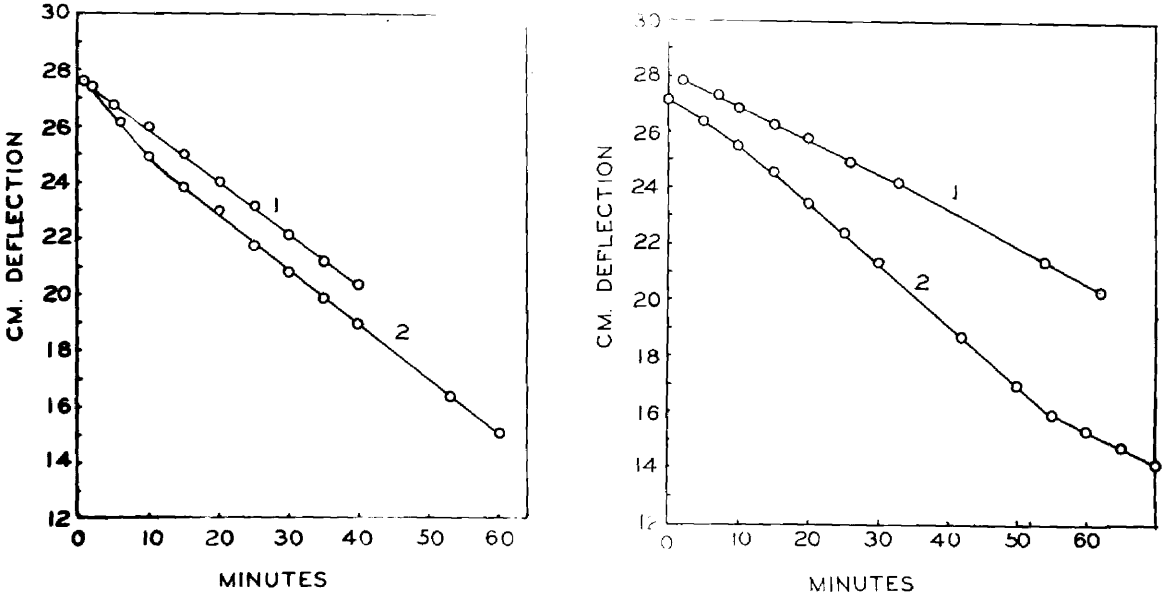


Fig. 14: The effect of KCN on respiration (80 hour coleoptile). Curve 1: Coleoptile treated during 25 minutes with $\frac{N}{3000}$ KCN, Curve 2, Oxygen consumption in nutrient solution.

Fig. 15: Same. (120 hour coleoptile)

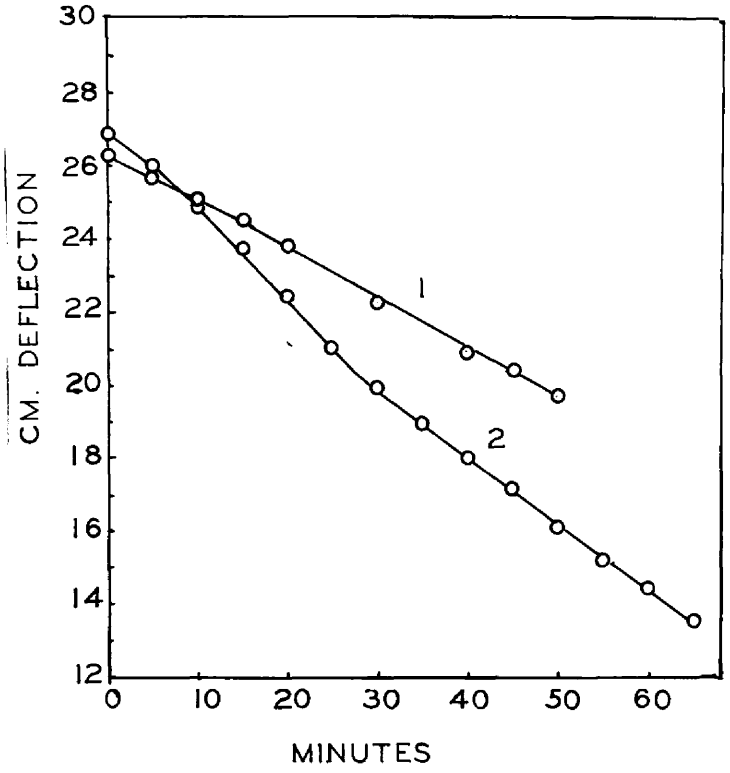


Fig. 16: Same as Figs. 14 & 15. $\frac{N}{100}$ KCN 105 hour coleoptile.

respiration by KCN parallels that of the reduced rate below the critical O_2 tension. This suggests that the system inhibited by low O_2 tension and the system inhibited by KCN are one and the same, although, of course, further work with other specific inhibitors is needed to corroborate this point further.

Since KCN inhibits protoplasmic streaming and respiration to the same degree it would be of interest to compare the effects of other substances on the two processes, the ultimate proof being effected by substances increasing the two processes to the same degree. However, as stated in Chapter II none of the substances used increased the streaming rate, and in view of the lengthy procedure involved in the determination of the inhibition of each of these substances in various concentrations on respiration this was not considered advisable here. There still remains, however, some doubt concerning the action of several of the substances used in Chapter II and this can be checked by respiration measurements, For example, dinitrophenol has been [illegible]. Moreover, it is of utmost interest at this time to compare the effect of auxin on streaming and respiration.

2-4-dinitrophenol is reported by Hohn (1937) to show a polarogram with 2 steps or flat portions falling in the lower region of the O_2 polarogram. Accordingly it was found that this substance affected the measurement of O_2 concentration measured here and like HCN would best be applied to the coleoptile outside of the electrode vessel.

This was carried out with concentrations from 1-100 mg./liter, and the results were found to agree with those in Chapter II. Some of the experiments are represented in Figures 17 and 18. Concentrations of 1 mg./liter had no effect on respiration independent of the time of exposure; 10 mg./liter up to 30 minutes exposure showed no effect, while 50 mg./liter at 30 minutes exposure showed marked inhibition of respiration. 100 mg./liter applied for 30 minutes completely stopped respiration. As in the case of the HCN experiments the streaming rate of the inhibited coleoptiles was measured at the close of the experiment and was found to be inhibited to the same degree as the respiration rate.

No polarogram has been reported for 3-indol acetic acid and it was found to have no effect on the O_2 polarogram in the low concentrations used (.01-100 mg./liter) in the following experiments. It could, then, be placed directly in the electrode cell and its effect determined immediately. Therefore, experiments were carried out in this way. The respiration rate in nutrient solution alone was determined for an hour or more, after which the solution was carefully withdrawn and replaced by nutrient solution + 3-indol acetic acid. Readings were taken again immediately after the dropping electrode was replaced. In all concentrations up to 10 mg./liter no effect of auxin was observed but in higher concentrations (25-100 mg./liter) inhibition occurred (Fig. 19). These results agree also with those in Chapter II.

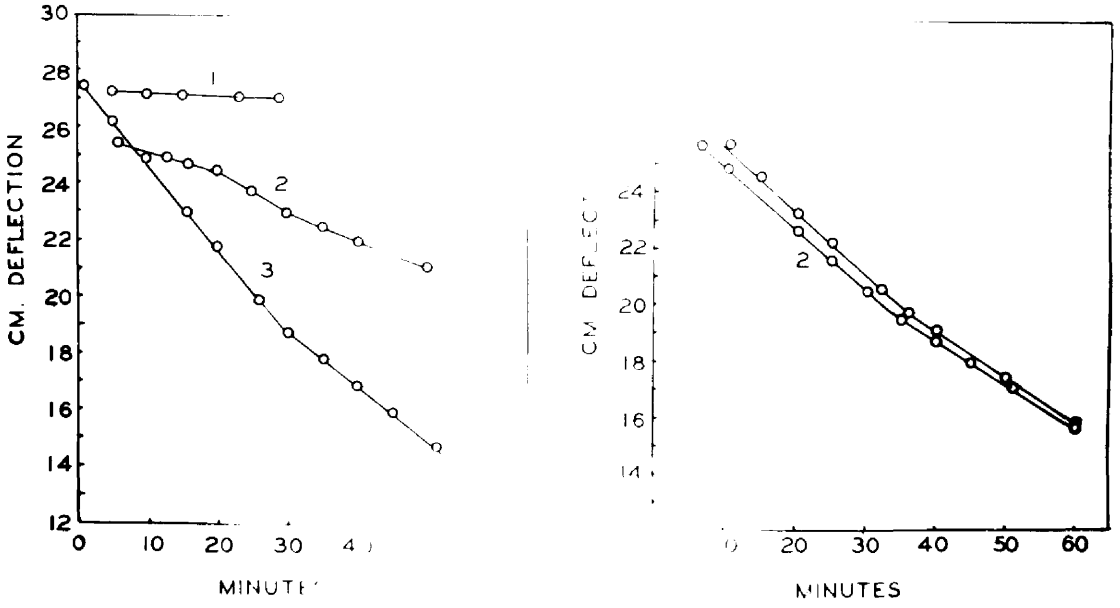


Fig. 17: The effect of 2-4-dinitrophenol on respiration. Curve 1: 100mg./l, Curve 2: 50mg./l, Curve 3: Oxygen consumption before treatment. Solutions applied during 25 minutes.

Fig. 18: Same as Fig. 17. Curve 1: 10mg./l. Curve 2: untreated.

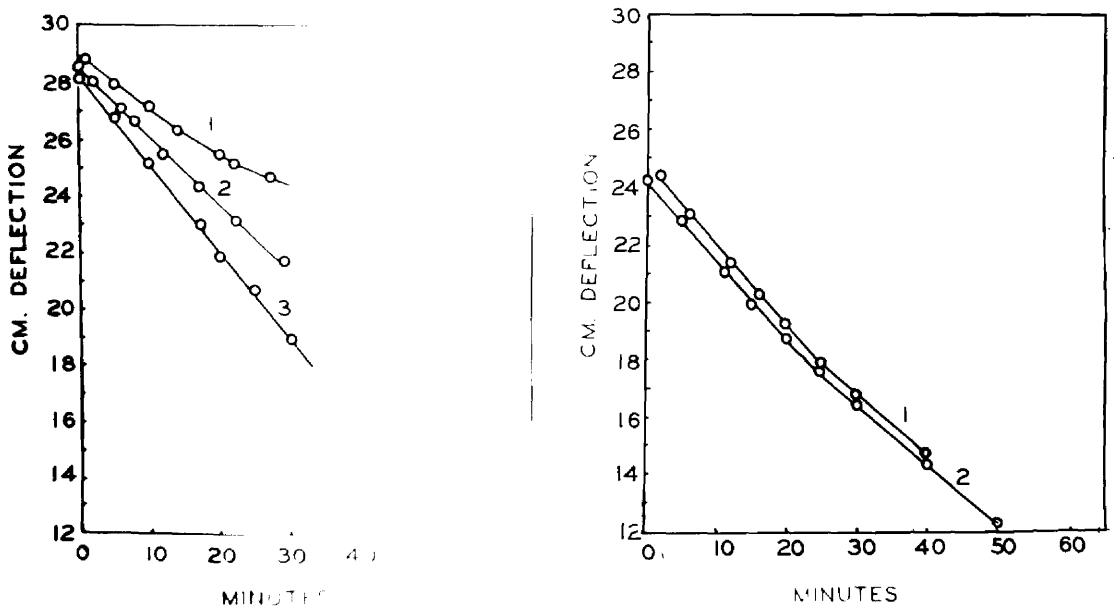


Fig. 19: The effect of 3-indol acetic acid on respiration. Curve 1: 50mg./l, Curve 2: 25mg./l, Curve 3: nutrient alone

Fig. 20: The effect of auxin + fructose (1%) on respiration. Curve 1: Auxin solution, Curve 2: Untreated solution alone.

Auxin + fructose (1 per cent) also showed no increase in respiration. (Fig. 20) Fructose is reducible at the cathode but its decomposition potential is beyond the range of the O_2 polarogram. It was found to have no effect on the latter, and therefore was applied to the coleoptile in the electrode cell with the auxin. Fructose alone was also found to have no effect on the coleoptile respiration.

DISCUSSION

The dropping mercury electrode, as has been shown, offers a remarkably accurate micro-method of determining the respiration rates of low respiring material such as the Avena coleoptile. It may be argued, however, that this method as used here depends upon a relatively inaccurate method of calibration. It is true that the Winkler method when used with such small samples is subject to no small error due to contamination by oxygen in the air during collection of these samples. Nevertheless, the calibration method was used here only for the purpose of following the straight line relationship between the galvanometer deflection and oxygen concentration. It was also used, of course, to determine approximately the oxygen concentrations corresponding to galvanometer deflection but not the absolute concentration of oxygen at any given deflection. This absolute concentration is not needed here since only relative differences are compared, and the sensitivity of the instrument is not decreased by this fact. Absolute

measurements might be obtained by using another method of calibration such as, for example, by carefully making up solutions of known oxygen concentration by saturating with oxygen under known partial pressures. The Winkler method, however, was found to suffice here.

There are other possibilities concerning the critical oxygen tension for respiration. It has not been determined, for example, whether or not this is due to the inhibition of the respiration of one tissue (i.e. parenchyma), the epidermis contributing to the remaining respiration. To determine this it would be necessary to remove the epidermis and study the respiration of the parenchyma tissue alone. In order to do this the difficulties introduced by tremendous wound surface must be overcome as well as those introduced by the availability of an excess amount of colloidal material from the wounded epidermal cells. In view of this difficulty no such experiments were carried out. However, indirect proof that this is not the case is provided by the observation that at low O_2 pressures the streaming of the parenchyma cells stops at the same time as that of the epidermal cells.

Bottelier (1937) has further studied the effect of oxygen tension on the rate of streaming. By a series of ingenuous calculations he has established a relationship between cell surface and streaming rate. However, all his calculations are based on the characteristics of those cells in the region about 5 mm. below the coleoptile

tip. If streaming rate and respiration as well, as he suggests, are dependent upon cell surface in this region the same relationship would be expected to hold for all other regions of the coleoptile. However, in this investigation no differences in the limiting effect of oxygen was found in the apical cells from that of the basal cells. For example, when a coleoptile is placed under a cover glass and time is allowed for the oxygen to become depleted the streaming is inhibited in all the coleoptile cells alike and not first in the tip and last in the base. Moreover, other differences than surface exist between the basal cells and the apical cells of the coleoptile. It is well known that pronounced differences in cell wall thickness occur between these two regions and this factor can be of as much importance in limiting oxygen uptake as the cell surface. Therefore, until further work can be carried out with respect to the effect of cell structure on this critical oxygen tension little more can be said.

A further relationship between the critical oxygen tension of respiration and that for streaming is brought out by Bottelier's early work (1934) concerning the effect of temperature on the rate of streaming. Bottelier found the temperature curve (streaming rate plotted against temperature) for old coleoptiles to consist of a Van't Hoff curve with a Q_5 of 1.33, while that for young coleoptiles was found to have a Q_5 of 1.05. For coleoptiles of

intermediate ages the temperature curve followed that of old coleoptiles until a certain temperature was reached, depending upon the age, and then suddenly would follow that for young coleoptiles. His conclusion was that streaming was contributed by two processes, one of which becomes limiting at a given temperature depending upon the age of the coleoptile. Since the process having a Q_5 of 1.05 points to the existance of a physical process he suggested that oxygen diffusion into the cell was the limiting process. Since water at higher temperatures contains less dissolved oxygen than water at lower temperatures this break in the curve can be attributed to the limiting effect of oxygen but no proof has been established that oxygen diffusion into the cell is a limiting factor. Since the relationship between the rate of streaming and oxygen concentration has not been determined by measuring streaming rates over a wide range of oxygen concentrations it cannot be said that the limiting oxygen concentration for streaming falls at the same point as the limiting concentration for respiration. Yet it is well known that the critical O_2 tension for streaming is higher for young coleoptiles than for old coleoptiles. Measurements of this type were not carried out here since a system must be used in which a solution of known oxygen concentration can be applied to the coleoptile during observation without contamination by oxygen from the air. Moreover, it cannot be definitely stated as to whether or not streaming at its limiting concentration is in hibited

to the same degree as is respiration. However, it was noticed during this investigation that a coleoptile (4cm.) placed under a cover glass soon is inhibited to a more or less constant rate when the oxygen is depleted to the extent that it becomes limiting. This inhibited rate appears to persist for some time before streaming actually stops. This phenomenon was not followed by careful measurements, however, since one cannot rule out the possibility of oxygen diffusion into the water under the cover glass from the air. Moreover, the oxygen in such a thin layer of water must have a very high diffusion resistance and the region surrounding the coleoptile may likely be depleted more rapidly than in the rest of the water. This factor, too, would introduce variabilities.

In view of the fact that respiration is inhibited by HCN to the same degree as is streaming and that the effect of dinitrophenol is similar it seems safe to make the assumption that streaming is dependent directly on respiration. It is realized, of course, as was mentioned before, that further work with other specific inhibitors for other reactions in the respiration (i.e. phenyl-urethane, carbon monoxide, etc.) must be carried out in order to establish this point. At any rate, it can be said that two respiration systems exist in the coleoptile, one of which is the cytochrome-indolphenol-oxidase system and the other some cyanide insensitive system such as is associated with the action of the yellow ferment or flavin enzyme.

Bonner (1936) reported an 80% decrease in respiration due to KCN which does not agree with the results reported here. However, it must be remembered that in Bonner's experiment (Wahrburg apparatus) the KCN was added to the preparation and allowed to remain in contact with it during the course of several hours. Since measurements were made over long periods a marked inhibition due to KCN would be expected since continued inhibition of the cyanide sensitive system might well suffice to bring about the death of the coleoptile. There is also the possibility of some secondary toxic effect occurring after such a long interval. If the action of KCN is to be interpreted as a specific poison of the indolphenol-oxidase it would be expected to occur over a relatively short period with the resulting inhibition of respiration occurring almost immediately. This is more characteristic of the other experiments on cyanide sensitive systems.

A limiting oxygen tension for respiration was also not reported by Bonner. This could not be expected, however, because even after prolonged determination with the Warburg the resulting decrease of oxygen partial pressure in the respiration vessel is far too low to deplete the oxygen in the solution surrounding the coleoptile to the extent that the critical oxygen tension (0.45 vol %) is reached. The polarographic method used here is far more favorable for such a study.

The failure to find the accelerating effect of auxin on respiration seems to indicate for certain that

there is no such effect of this substance in the process of elongation. Respiration increase by auxin has been reported by Pratt (1938) for the developing embryo of the oat plant but this does not mean that such an increase must occur in the coleoptile, since here the action of auxin is strictly one of elongation. Moreover, the concentrations used (Pratt) were abnormally high and sufficient to limit the growth of the embryo. Further work must be done with physiological concentrations on other material.

Since protoplasmic streaming can be associated with auxin transport the next step in this investigation is obviously the determination and comparison of these same substances on transport. This will be taken up in the next chapter.

CHAPTER IV

FACTORS INFLUENCING AUXIN TRANSPORT

The effect of various solutions or substances, with the exception of Clark's work with sodium glycocholate and saponin, on the transport of Avena coleoptile sections has been little worked out. Van der Wey (1934) studied the effect of ether narcosis, but further work with other substances was not carried out. As has been mentioned before, the similarity of the effect of KCN and dinitrophenol on respiration and protoplasmic streaming suggests, if the protoplasmic streaming period of transport is valid, a similar effect of these substances on transport. Experiments to determine this effect are carried out in the following.

METHOD

The original transport technique as used by Van der Wey,(1934) was followed in principle with several modifications. This procedure in general is as follows:

Agar blocks are soaked for one hour in auxin solution (3 indole acetic acid) of known concentration and applied to the apical cut surface of coleoptile sections. To the basal cut surface of these same coleoptile sections plain agar blocks are applied. The whole series of sections is then allowed to stand in a vertical position, basal ends downward, for the period for which the amount of auxin transported is determined. Both the upper and lower blocks

are then analysed for their auxin content by the usual Avena technique.

In order to eliminate the danger of upsetting the sections and blocks during the experiment the series of sections was laid on a glass slide and the blocks applied to each end. The plate was then placed in a vertical position such that the bases of the sections were downward. Capillary water continuity between the sections and the slide was prevented by a very narrow strip of filter paper separating the sections and the slide.

If significant results are to be obtained with the transport technique three related conditions must be carefully observed and controlled. These include (1) the length of the sections; (2) the interval over which transport is measured; and (3) the concentration of auxin applied to the upper blocks.

If the sections are too long most of the auxin applied will be in the section itself during the transport interval and much of it will be used in the process of elongation of the section. Hence little will be transported into the lower blocks.

If the transport interval is too long the auxin transported by both the control and experimental sections will all have reached the lower blocks and the results will show no difference in transport rate.

The choice of amount of auxin applied in the upper blocks requires even more consideration. Since there

exists an upper limit of auxin concentration to which the test plants will respond, the concentration in the upper blocks must never be so high that the amount transported will reach this concentration either in the control or experimental sections.

These conditions have been carefully worked out for the following experiments and are as given in the tables.

The substances studied for their effect on transport (2-4-dinitrophenol and KCN) were applied to the coleoptile sections by infiltration. The sections were then removed and all water films or continuities removed by blocking. The transport of these sections was then measured as outlined. Control sections were infiltrated with water.

RESULTS AND DISCUSSION

The results of experiments with HCN are shown in Table I. It will be seen that the degree of transport inhibition shows a marked similarity to the degree of streaming and respiration inhibition found in Chapters II and III. At the close of the experiment the sections were examined for their streaming rate and degree of turgidity or flaccidity and these observations are also given in the table. The inhibition of streaming and transport in the highest concentration appears to be much greater than would be expected, however, it must be remembered that the sections are in continued contact with HCN at this high concentration

over the full period of the experiment (about 2 hours). It is not unlikely, then, that some secondary toxic effect would bring about a permanent inhibition and even cessation of streaming. The flaccidity of these sections at the end of the experiment also points to some such action. Action of the lower concentration on transport, however, agrees favorably with its action on streaming and respiration.

In Table II is shown the effect of dinitrophenol on transport. Here again is found the same degree of inhibition of transport as that of streaming and respiration. The higher concentration (100 mg./liter) does not kill the cells as in the case of KCN in high concentration, but the transport is completely stopped. The concentration of 10 mg./liter, by its continued inhibition without redovery, decreased transport by about per cent. Completely in accord with its effect on streaming and respiration, 1 mg./liter had no effect on the transport.

These results bring out clearly the relationship between protoplasmic streaming and transport, since in all cases the transport was decreased to the same extent as was streaming.

In all cases with each substance the transport rate was decreased by the same concentrations and to the same degree as was the protoplasmic streaming rate. Hence the results bring out clearly the relationship between protoplasmic streaming and transport.

Table I

Effect of KCN

Length of cylinders : 5 mm., 12 cylinders per conc.
Exposure time to KCN : 40 minutes
Transported solution : 10 mg. indol acetic acid per liter
Transport time : 75 minutes
Temperature : 23.5° C

Coleoptile zone 2 - 7 mm. from apex

Conc. KCN	N/100	N/3000	Control
Upper blocks	8.9 ± .8	7.8 ± 1.5	6.3 ± .4
Lower blocks	8.0 ± .8	9.2 ± 2.3	25.4 ± .9

Coleoptile zone 12 - 17 mm. from apex

Upper blocks	9.8 ± 1.5	6.0 ± 3.0	8.6 ± .4
Lower blocks	13.0 ± 3.6	15.8 ± 2.2	29.0 ± .3
Condition of Cylinders	somewhat flaccid	turgid	turgid

Table II

Effect of dinitrophenol

Experimental conditions the same

Coleoptile zone 2 - 7 mm. from apex

Conc.DNP in mg./l	1	10	100	Control
Upper blocks	12.8 ± 3.3	14.8 ± 3.1	15.2 ± 3.6	14.3 ± 1.6
Lower blocks	28.7 ± 4.5	18.8 ± 2.5	0	25.3 ± 3.7

Coleoptile zone 12 - 17 mm. from apex

Upper blocks	13.5 ± 1.5	13.0 ± 3.6	13.4 ± 3.1	12.7 ± 1.2
Lower blocks	17.6 ± 3.1	16.4 ± 3.6	5.5 ± 1.3	19.4 ± 3.5
Condition of Cylinders	turgid	somewhat flaccid	flaccid	turgid

CHAPTER V

SUMMARY AND DISCUSSION

SUMMARY OF RESULTS

1. A decided improvement in the conventional method of observation of protoplasmic streaming in the Avena coleoptile has been made through the use of unilateral dark field illumination. With this method more reliable measurements can be obtained with greater ease of observation than with previous methods.

2. An entirely objective photographic recording method of protoplasmic streaming rate has been devised depending upon the length of the streak on a film made by a moving particle during a given exposure time. Special difficulties must be overcome, however, in order to use this method with the Avena coleoptile under all conditions.

3. The effect of ethyl ether on protoplasmic streaming was determined. No increase in rate was caused by these substances, but marked inhibition was found in higher concentrations. (10 g./liter - 32 g./liter)

4. No marked increase in the rate of streaming by auxin in concentration of .005 - 10 mg./liter could be found. Above this range inhibition was observed.

5. The effect of 2-4-dinitrophenol on streaming was found to be permanent and toxic in concentrations of 50 and 100 mg./liter, with no effect at 1 mg./liter.

6. No increase of streaming by methylene blue from 10^{-5} molar - 10^{-2} molar concentration could be sh own. Above this range inhibition occurred.

7. KCN was found to inhibit the streaming process only about 50% even in concentrations as high as $\frac{N}{100}$.

8. The dropping mercury electrode has been adapted to the measurement of the respiration of a single coleoptile section.

9. There occurs at a definite low oxygen concentration a critical point in the respiration proceas at which a new and lower rate of respiration persists. This lower rate is about 50% of the normal rate.

10. This limiting concentration is lower for old coleoptiles than for young coleoptiles.

11. HCN limits the rate of respiration to the same extent that it limits the rate of streaming. The inhibited rate of respiration is about the same as that which is brought about by low O_2 tension.

12. 2-4-dinitrophenol inhibits the rate of respiration to the same degree that it limits streaming.

13. Auxin alone or in the presence of fructose has no effect on respiration except in the higher concentrations (25 - 100 mg./liter) where it inhibits respiration.

14. KCN limits the transport of auxin to the same degree and in the same concentrations that it limits protoplasmic streaming and respiration.

15. 2-4-dinitrophenol decreases the transport of auxin to the same extent and in the same concentration that it decreases protoplasmic streaming and respiration.

DISCUSSION OF RESULTS

The relation between the three processes, protoplasmic streaming, respiration, and transport, has been pointed out previously from the results of Chapters II, III, and IV. All three processes have been shown to be limited to the same extent by the same concentrations of KCN. They are therefore all controlled by the process specifically inhibited by this substance, namely, that catalyzed by the cytochrome-indophenol oxidase system. It is clear from this, then, that transport is controlled by streaming and that streaming is controlled by respiration and that any factor affecting respiration should similarly affect transport via streaming.

As a further proof, 2-4-dinitrophenol is shown to inhibit the three processes alike although its action appears to be more associated with the streaming process.

The limiting effect of oxygen and the occurrence of a definite critical oxygen tension for respiration, lower for old coleoptiles than for young coleoptiles, further establishes the link between streaming and respiration, since it parallels the effect of low oxygen tension on the streaming rate of old and young coleoptiles. Careful experiments on the effect of low oxygen pressures on transport would undoubtedly show a similar critical oxygen pressure for transport, lower for old coleoptiles and higher for young coleoptiles. Such a study, however, is a complete investigation in itself and was not included here.

In conclusion, then, it may be stated that all the results fit the following picture for auxin transport. Auxin is transported through transverse cell membranes only from tip to base. This is a polar property specific to the membranes and the factors responsible for it are little known. As the auxin enters the apical end of a cell through such membranes, by virtue of its gradient from tip to base, it is carried by the protoplasmic streaming and is equally distributed throughout the cell. Having thus traversed the gap from transverse wall to transverse wall in a much shorter time than it could by diffusion alone it proceeds down into the next lower cell and there is carried by streaming in the same manner. A decrease in the streaming rate will prolong the period during which auxin is distributed throughcut the cell and in the case of cessation of the streaming this distribution of auxin in the protoplasm will depend upon diffusion alone.

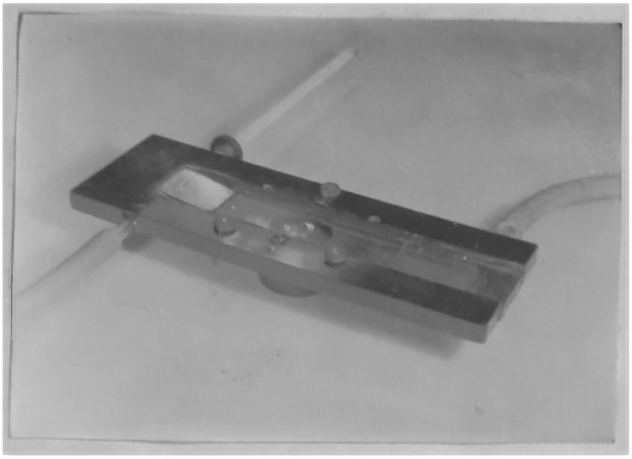


Fig.21. Constant temperature stage used in observing the protoplasmic streaming

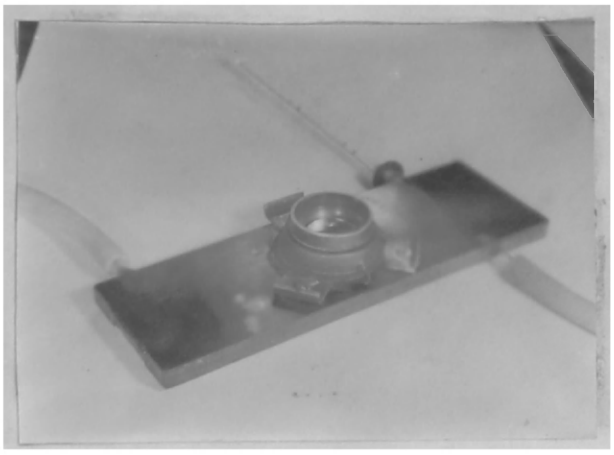
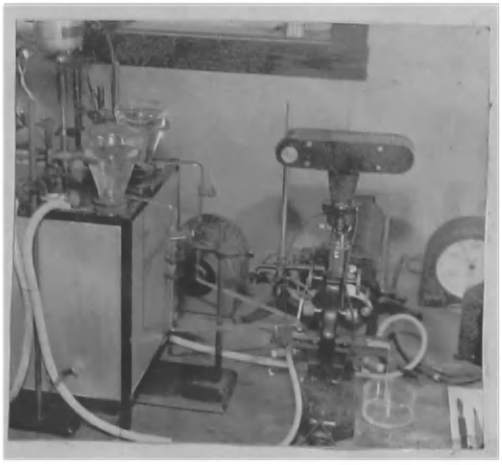


Fig.22. Same, from below shows condenser attachment and the arrangement for centering on the microscope.

Fig.23. Apparatus used in observing and recording changes in the rate of the protoplasmic streaming.



Fig,24. Close-up of same showing arrangement for flowing solutions under the cover glass.

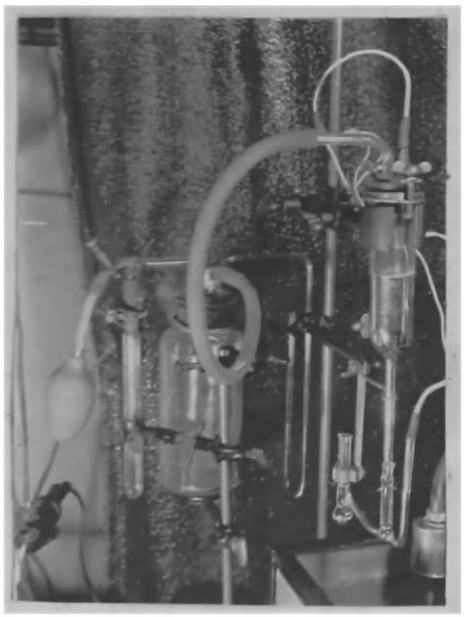


Fig.25. Dropping mercury electrode assembly showing pressure device.

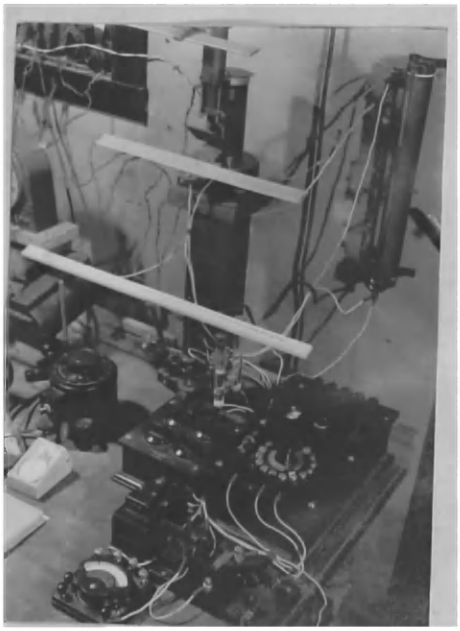


Fig.26. The circuit and galvanometer arrangement for same.

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