GROWTH SUBSTANCE AND CELL ELONGATION

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-Foreword.-

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TABLE OF CONTENTS.

PART I. The Study of Cell Elongation before the Discovery of Growth Substance

a) Introduction 1

b) Osmotic Pressure of the Cell Sap 2

c) Elasticity 4

d) Plasticity 4

e) Permeability 5

f) Intussusception 6

g) Imbibition Pressure 7

PART II. The Study of Cell Elongation Since the Discovery of Growth Substance

a) Discovery and Isolation of Growth Substance 9

b) The Avena Seedling and Coleoptile 10

c) The General Relation of Growth Substance to the Coleoptile 11

d) Growth Substance and the Deformability of the Coleoptile 13

e) Objects of the Present Work 15

PART III. A General Discussion of the Materials and Methods

a) Plants 17

b) Growth Substance 17

c) Units 18

PART IV. The Hormonal Nature of Growth Substance

a) Introduction 19

b) Methods 20

c) The Amount of Growth Substance Entering the Plant 21

d) The Amount of Growth Corresponding to a Given Amount of Growth Substance at 27°. 22

PART IV. The Hormonal Nature of Growth Substance (cont.)

e) The Amount of Growth Corresponding to a Given Amount of Growth Substance at 15°. 30

f) The Composition of the Cell Wall. 31

g) The Volume of the Cell Walls 33

h) The Ratio of Cell Wall Constituents Produced to Growth Substance Used. 35

i) Conclusions 40

PART V. The Relation of Wall Formation to Cell Elongation

a) Introduction 52

b) Methods 53

c) Experimental Results 54

d) Conclusions 55

PART VI. Conditions Governing the Elongation of Excised Coleoptile Sections.

a) Introduction 59

b) Methods 60

c) Elongation in Water Alone 61

d) Stirring 62

e) Former Position of Section in the Coleoptile 62

f) Growth Substance Concentration 63

g) Fructose 64

h) Inhibition of the Aerobic Metabolism 64

i) Conclusions 67

PART VII. The Relation of Hydrogen Ions to Elongation.

a) Acid Growth and Acid Curvatures 74

b) The Nature of the Phenomena 76

c) Acidity of the Coleoptile during Growth 83

d) Dependence of Acid Growth on Growth Substance 87

e) Discussion and Conclusions 93

PART VIII. Growth and Respiration.

a) Introduction 104

b) Methods 111

c) The Coleoptile as a Whole

Elongation and Respiration during the Grand Period 113

Distribution of Growth and Respiration in the Coleoptile 117

d) The Rapidly Growing Zone

Changes of Growth and Respiration with Time 120

Effect of Cyanide upon Growth and Respiration 121

Action of Growth Substance upon Growth and upon Respiration 122

Effect of Temperature on the two processes 124

Characteristics of Growth and Non-growth Respiration 128

The Action of Growth Substance 132

e) Discussion 136

f) Summary 138

CONCLUSIONS 152

LITERATURE 154

PART I.

THE STUDY OF CELL ELONGATION BEFORE THE DISCOVERY OF GROWTH SUBSTANCE

a. Introduction

The study of cell elongation is as old as the study of plant growth for the reason that the visible increase in size of a plant organ is principally due to cell elongation. The first fruitful theory, however, concerning the nature of the processes and forces involved was enunciated by Sachs (1882). According to Sachs, the cell wall is elastically stretched, by the pressure of the cell contents. Due to this stretching, the particles of which the wall is composed become further apart and new ones are then set down between them. This relieves the pressure of the wall and the cycle repeats itself ad infinitum or as long as the cell contents can exert sufficient pressure on the wall. The theory contains the essential points, a) elastic stretching of the cell wall by internal pressure, b) intussusception of new wall material. The first point was regarded as substantiated by the fact that as found by DeVries (1874), Laurent (1885) and others, the amount of elastic stretching of growing tissues is greatest at the time of fastest growth. The second point was based upon the work of Nägeli (1858) who had concluded that the growth of cell walls as well as of starch grains is by intussusception. It may be said at once, however, that the theory of Sachs has been fruitful principally because of the investigations which have been instigated for its attack or defence. Schwendener and Krabbe (1895) failed to find the proportionality between turgor stretching and growth rate

described by DeVries, and Klebs (1886) could not find the least elastic extension in growing filaments of _Zygnema._ Wortmann (1887) and Noll (1887) found as did Sachs, that elastic stretching is essential to growth. Concerning the intussusception of new material in the cell wall also, a divided opinion is to be found in the older literature. Wortmann found that slowly growing portions such as the upper side of a geotropically stimulated stem rapidly thicken their cell walls, and concluded that thickening and consequent decrease of elasticity is the cause of the eventual cessation of growth. Krabbe (1887) and Schwendener and Krabbe (1893) went so far as to claim that all growth is due to an active intussusception without the intervention of turgor stretching. Sufficient has been said to make it clear that from the beginning there has been a considerable variance between the views of the several investigators. A methodical examination of the general theories which have been put forth will be made and an attempt made to evaluate each.

b. Osmotic Pressure of the Cell Sap

The primary cause of cell elongation is elastic stretching due to the osmotic pressure. It is necessary to make it clear that altho cell elongation in the normally growing plant may be due to the maintenance of a given property, for example osmotic pressure, at a sufficiently high value, nevertheless it is technically often more convenient to work with increases in growth rate and increases in the given property, for example, of osmotic pressure. For this reason the convex and concave sides of tropistically stimulated organs have been much used. Altho there might once have been objection to this procedure, there cannot be now, since as is clear

from our present knowledge of tropisms, there is no fundamental difference between tropistic growth response and normal cell elongation.

DeVries believed to have found that the osmotic value of the cell sap is higher in faster growing regions. Schley (1913, 1920) in similar experiments came to the same conclusion. Krauss (1882), ~~Meeschen (1909) and~~ Phillips (1920) failed to find this increase. Pfeffer (1892) even found that hindering of elongation brought about an increase of osmotic value and this was supported by Pringsheim (1906). More recently Warner (1928) working with geotropically stimulated organs has shown quite definitely that there is an increase of sugar on the concave side, but that there is also an increase of acids on on the convex side. Borowikow (1913, 1914) found that variations of temperature or of acidity which decreased the growth rate of cells, correspondingly increased their osmotic value. The inverse relation between growth rate and osmotic value has been supported by Reed (1925), and Fernald (1925, by cyroscopic measurements. Ursprung and Blum (1924) investigated the matter somewhat further and obtained finally a conclusive answer. They found that altho the two sides of a geotropically stimulated organ possess almost the same osmotic value, the convex side has a much higher suction force, which can then only be interpreted as due to lower wall resistance. It is clear from their work that increases of osmotic value cannot be the direct cause of increases of growth rate.

c. Elasticity

The primary cause of growth is an increase in the elasticity of the cell wall. It has already been pointed out that Klebs (loc. cit.) failed to find any elastic extensibility in growing cells. Wortmann (loc. cit.), however, held that the gradual thickening of the cell wall reduced its elasticity to the point where elongation by the pressure of the cell contents could no longer take place, i.e. that decreases in elasticity decrease growth. Noll (loc. cit.) supposed that under the influence of the protoplasm the wall was kept continually in an elastic condition to permit growth. Increases of growth rate would then be due to increases of elasticity. Overbeck (1926) studied geotropically stimulated roots whose curvatures were prevented. These roots when freed gave extraordinarily swift "schnell Krümmungen", which, however, were almost completely removed by immediate plasmolysis. By a later plasmolysis only a small portion of the curvature could be removed. He concluded that during geotropic stimulation a one sided increase in elasticity had occurred which permitted the "schnell Krümmung" and that the curvature was then gradually "fixed". DeHaas (1928) found that normally growing Avena coleoptiles possessed higher elasticity than coleoptiles whose growth had been slowed down by decapitation (see Part II). These cases show that high elasticity often, but not always, accompanies fast growth. Heyn (1931) has finally settled the question. He has shown that in the Avena coleoptile and therefore probably in other cases increases in elasticity are results rather than primary causes of growth. This will be discussed in detail in Part II.

d. Plasticity

The primary cause of growth is a plastic stretching of the cell wall. Laurent(loc.cit.) postulated an enzyme action upon the cell wall which should result in a plastic condition. He was, however, unable to fortify his position by experiment. Klebs(loc.cit.) was willing to admit that the action of the protoplasm might increase the wall plasticity and showed that in algal filaments the elongation was almost entirely irreversible. Ziegenspeck(1920) found great plasticity and little elasticity in root hairs. Pfeffer(1903) considered a "diminution of the cohesion of the cell wall" to be neccessary for elongation. He showed directly that this softening was a result of the action of the protoplasm. It is again, however, due to the work of Heyn(1931) and of Heyn and Van Overbeek(1931) that a final solution of the question of plastic versus elastic stretching has been finally obtained. This work will be discussed in part II.

d. Permeability

The primary cause of growth is an increase in the permeability of the cell membranes. Several investigators have expressed the opinion that increases in cell permeability should increase growth rate. Tröndle (1910-18) thought that in tropistic reactions the primary effect of the stimulus was one upon permeability, and this view has been supported by Small (1919), by Brauner (1924) and by VanDillewijn (1927). It is well established that large changes of permeability result from the action of various stimuli. For example light increases permeability according to the work of Blackman and Payne (1918), Hoagland and Davis (1923), and Brauner (1924), to mention only a few works less open to objection than those of Lepeschkin (1909) and Tröndle (1910) which are usually quoted. Light probably increases the water permeability of the pulvinal cells of Phaseolus, according to M. Brauner (1932). Movements of the leaves of sensitive plants and of sensitive stamens as those of Sparmannia are quite certainly accompanied by increase of the protoplasmic permeability and the consequent expression of cell sap (Bünning, 1933 a, b). It is therefore not at all unlikely that changes of permeability do occur both in normal growth and in tropisms. Such changes would be of value for cell elongation only if, for example, water permeability were the factor limiting elongation, and there is no reason to believe that this is, in general, the case. In Part VI it will be shown that it can hardly be the case in the Avena coleoptile. Changes in the direction of permitting an exosmosis as in the case of stamens would be actually detrimental to cell elongation.

e. Intussusception

<u>The primary cause of growth is an "active wall growth"</u>. This phrase has meant in general growth by forcing of <u>new</u> wall material in between the particles already deposited. As pointed out by Pfeffer (loc. cit.) and by Strassburger (1898) not all growth could be of this kind since wall growth is in many cases by apposition, in which case increase in surface extent must be by some kind of stretching. Nägeli (loc. cit.) first put forward the theory that growth of the cell wall is by intussusception. This was in part based upon his erroneous belief that the growth of starch grains is of the same kind. Pfeffer cites some cases which seem to be certainly the result of some "active growth", for example the wrinkling of cell walls in cells no longer stretched. Krabbe (loc. cit.) supported active growth without giving it any strong experimental verification. Ursprung and Blum (loc. cit.) have advanced active growth as important, principally because they failed to see how turgor, at its minimum in rapidly growing cells, could have any bearing upon elongation. Rentschler (1929) similarly adopted it as a last alternative. More recently Söding (1934) and Strugger (1932, 1933, 1934) have supported active growth, but in both cases this is merely an opinion based on no convincing evidence. Against the general importance of intussusception may be cited the work of Pringsheim (1931) and the work of Heyn which has shown that cell elongation is possible by an irreversible increase of cell wall area in the absence of any possibility of active growth. It is still necessary, however, to demonstrate in each case whether or not the formation of new cell wall is an integral part of the elongation process.

f. Imbibition Pressure

The primary cause of growth is the imbibition pressure of the protoplasm. Loyd (1916), Calabeck (1927), Ulehla and Moravek (1922), McDougal (1925), Walter (1924), and most recently Strugger (1934) have supported various aspects of the general theory that increases of the imbibition pressure of the protoplasm or other biocolloids are the primary causes of increases in cell elongation. This theory has often taken the form of postulation of a mechanism for the increase of the pressure of the cell contents on the wall without an increase in the osmotic value of the cell. It is clear, however, and it has been emphasized by Walter (loc. cit.), that the vapor pressure of water in equilibrium with the cell sap will under all conditions be equal to that in equilibrium with the protoplasm. Otherwise water will pass from one to the other until such an equilibrium is established. Moreover the imbibition pressure which the protoplasm exerts on the cell wall must be exactly equal to that exerted by the cell sap on the protoplasm and to the pressure with which the cell wall constrains the cell contents. Consider then what result an increase in imbibition pressure of the protoplasm might have. So far as increased uptake of water by the cell is concerned, water will be taken up until the protoplasm is again in equilibrium with the cell sap. In general for cells in the elongation stage and hence with very small amounts of protoplasm, this amount of water is negligible, and hence the elongation which it can bring about is negligible. There remains, however, the possibility that increase in the hydration of the protoplasm may influence, for example, the water permeability. This has been suggested

by Walter on the basis of the fact that Borowikow (loc. cit.) found the same effect of ionic lyotropic series upon growth of Helianthus hypocotyls as is commonly found for effects upon permeability. DeHaan (1933), Dellingshausen (1933), and Forster (1933) have some evidence to show that increases in protoplasmic hydration increase permeability both to water and to salts. It has been already pointed out, however, that there is as yet no good reason to believe that effects upon permeability have a direct relation to cell elongation.

Van de Sande Bakhuysen (1930) has advanced the opinion that hydration of the cell wall as well as of the protoplasm is of importance in cell elongation. It is possible to visualize an effect of hydration upon the cohesive properties of the cell wall and hence upon its plastic stretching, but as yet such an effect and its direct relation to cell elongation has not been demonstrated.

There can be no doubt but that some of the changes of growth rate which have been interpreted as due to changes in the hydration of the protoplasm have been actually due to changes in the actual water supply. This may be the case in the work of Walter (1924).

One of the principal factors which should influence protoplasmic imbibition is the pH of the protoplasm; it will be shown in Part VI that so far as the Avena coleoptile is concerned the effect of pH upon growth rate is not due to an effect upon protoplasmic imbibition. Changes of imbibition pressure of the protoplasm cannot therefore be of general significance in the control of growth.

PART II.

THE STUDY OF CELL ELONGATION SINCE THE DISCOVERY OF GROWTH SUBSTANCE

a. Discovery and Isolation of Growth Substance

The study of cell elongation has taken on a new aspect since the isolation by Went (1928) of a special substance whose presence in the plant cell is essential to elongation. The existence of special substances for the regulation of plant growth was first advanced by Duhamel (1758). Sachs (1879) brought forth a long list of facts indicating the existence of a special flower forming, root forming, leaf forming etc. substances, and proposed that correlation, i.e. the effect of one part on the growth of another, is in general mediated by diffusible substances. Sachs was, however, unable to supply any firm experimental evidence as to their existence. Haberlandt (1913-1919) went a step further. He demonstrated that a substance is stored in the leptome of potato tubers which is able to diffuse into tuber sections without leptome and bring about cell division there. Ricca (1916) showed that a substance which can pass through a tube filled with water controls the response of leaves in *Mimosa.* The idea of special substances was therefore not entirely new even in 1910 when Boysen Jensen proposed the existence of such a substance for the control of cell elongation, especially in the *Avena* coleoptile. Paal (1914, 1919) soon established the fact of correlation, with respect to elongation, by a diffusible substance. F. Went (loc. cit.) worked out a method of quantitative determination and obtained the substance free of the plant, and Kögl, Haagen Smit, and Erxleben (1933) prepared the substance in the crystalline form.

b. The Avena Seedling and Coleoptile

It has been shown by a variety of workers (Cholodny, 1927; Söding, 1932; Nielsen, 1930; Boysen-Jensen, 1931; Laibach, 1933; Van der Wey, 1933) that this "growth" substance or one of similar physiological action is widely distributed through the plant kingdom, probably universally. However, the following discussion will limit itself to the *Avena* coleoptile with which the greater part of the work has been done. Before embarking upon a detailed discussion of the growth of this organ, it would be well, however, to give a brief survey of the morphology and anatomy of the *Avena* embryo, seedling, and especially of the coleoptile. Reference may be made to the work of Avery (1928, 1930) who has investigated the anatomy in some detail.

The embryo is at the base of the seed, rather at one side and opposite the groove. The embryonic axis consists at the upper end of short internodes bearing whorls of leaf primordia. Below this is the insertion of the scutellum or cotyledon. The embryonic leaves and growing point are surrounded by the conical coleoptile. At the scutellar node are the buds of two lateral roots. Below the scutellar node is the primary root and its coleorhiza. A large branched procambial bundle proceeds from the scutellum to the scutellar node and hence into the stele of the embryonic axis. The vascular system of the coleoptile consists of two lateral bundles arising from the stele. After germination (Fig. I) the root consists of the usual exarch vascular system with one large central metaxylem vessel. At the scutellar node there is an abrupt transition to an endarch vascular system. The region between the coleoptile insertion and the scutellar insertion, commonly known as the mesocotyl is, then, of an

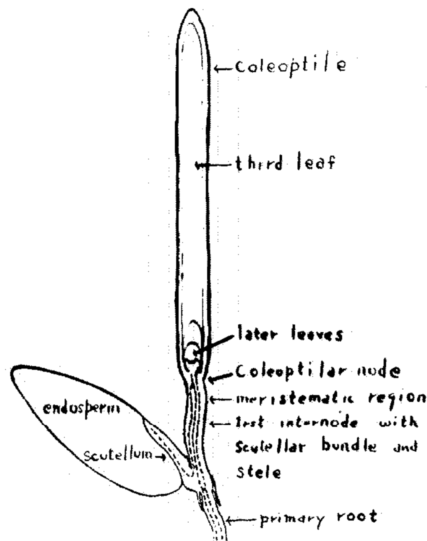

Figure 1.Radial Section through Col-
eoptile and Seed.

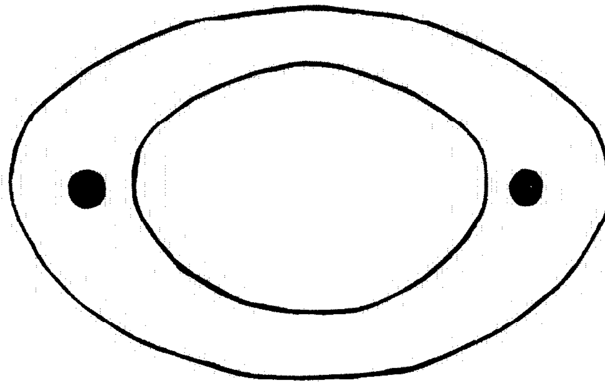

Figure 2.Cross Section through Coleoptile
Showing Location of Vascular Bundles.

essentially stem structure and is to be regarded as the first internode. A meristematic region at the top of this internode causes its elongation and at the same time elongates both the scutellar bundle and the stele, with the result that the two run parallel in the mature internode. Only at the level of coleoptilar insertion does the scutellar bundle enter the stele in the seedling. The coleoptilar bundles diverge from the stele at the point where the scutellar bundle turns down into it. The vascular bundles of the coleoptile are small with few xylem groups and comparatively well developed phloem. A bud is in general present in the axil of the coleoptile, which is therefore to be considered as a leaf. The leaves within the coleoptile are supplied with bundles in part from the stele and in part from the scutellar insertion.

The coleoptile is elliptical in cross section (Fig. II) with the long axis perpendicular to the plane of the seed. It is, of course, hollow and consists in general of about six cell layers. The innermost and outermost layers are of somewhat smaller cells than those of the inner parenchyma. The elongation of the coleoptile, from a mm. or less, to 40 or 50 mm., is unaccompanied by cell division (Tetley and Priestley, 1927). During this time also the diameter of the coleoptile changes but little. In the *Avena* coleoptile one has then a relatively pure example of growth by cell elongation.

c. General Relation of Growth Substance to the Coleoptile

In relation to growth and growth substance in the coleoptile the following facts have been established:

1) The growth substance is produced in the tip of the coleoptile and proceeds downwards toward the base. (Paal, 1913, 1919; Dolk, 1930; Went, 1928)

2) If the tip is removed, the top of the remaining stump commences to produce growth substance after the lapse of a definite time (Dolk, 1930; Tsi Tung Li, 1930). This has been called "physiological regeneration".

3) If, after decapitation and reappearance of growth substance in the upper zones, these zones are also removed the growth of the coleoptile falls practically to zero (Dolk, 1930).

4) Application of growth substance to the lower zones of old coleoptiles which have stopped growing, causes, under certain conditions, the recommencement of growth (Went, 1928).

5) The response of the coleoptile to phototropic and geotropic stimuli is conditioned by the presence of growth substance (Dolk, 1926, 1930; Went, 1928; DuBuy, 1933). After decapitation the ability of the upper zones to react to phototropic and geotropic stimuli is lost but returns at the same time as the new growth substance.

From 3, 4, and 5 it has been fairly concluded that the growth substance is not merely an accelerator of growth but that without it no growth can occur.

6) Growth substance disappears continuously during growth. Only a small portion of the amount produced by the tip can be recovered from the lower zones.

7) The movement of growth substance is a polar phenomenon (Went, 1928; Van der Wey, 1932). In inverted coleoptile cylinders no downward transport occurs.

8) The application of growth substance to decapitated coleoptiles results in an increase of their deformability (Heyn, 1931).

9) The one sided application of agar blocks containing growth substance to the stumps of decapitated coleoptiles causes a growth curvature whose magnitude is over a considerable range proportional to the amount of growth substance in the agar block (Went, 1928). This is the basis of the quantitative growth substance test (Van der Wey, 1932; Nielsen, 1930; Dolk and Thimann, 1932).

10) The growth substance is not an enzyme but a thermostable, lipoid soluble, unsaturated acid of molecular weight 328, and having the formula $C_{18}H_{32}O_5$.°

These are the essential points with respect to the relation of growth substance and growth. More detailed discussions of various aspects will be given in the later sections.

d. Growth Substance and the Deformability of the Coleoptile

The problems of cell elongation may now be attacked through an examination of the properties of the coleoptile which are altered under the influence of growth substance. The work of Heyn (1931), Heyn and Van Overbeek (1931) and of Söding (1929, 1931) has shown that some time after a coleoptile is given growth substance the elasticity and plasticity of the coleoptile are increased. The fundamental experiment is this: comparable sets of coleoptiles are more or less freed of growth substance by allowing them to stand for two hours after decapitation. One set is then supplied with growth substance and the other set not. The growth substance is allowed to act for some time, say two hours; the coleoptiles are plasmolized to remove whatever resistance might be due to the protoplasm, and the extensibility under an applied force determined. Heyn found that both the reversible and irreversible extensibility are increased after the action of growth substance and accompanying growth. He was able to go further than this, however, by giving coleoptiles growth substance but no water supply. In this case the elasticity remained relatively constant. This he considered to show that increases in elasticity are the result of elongation and that the primary action of growth substance upon the coleoptile is in increasing plasticity.

°Footnote: Later work of Kögl has shown that one or more closely related substances may also be active.

This experiment is of importance because it shows that increases in elasticity are principally the result of growth, not the cause.

Söding independently at about the same time and using somewhat similar methods came also to the conclusion that more rapidly growing coleoptiles have more extensible cell walls than do more slowly growing ones.

The increase in deformability by growth substance is well illustrated by experiments in which weights are hung upon the ends of of horizontally placed turgid coleoptiles. Under the conditions of Heyn and Van Overbeek the *reversible* bending was slightly affected, the *irreversible* greatly affected, by the presence of growth substance. This experiment was also done independently by Söding.

Note: The turgescent coleoptiles used in bending experiments have the formal requirements of growth (according to Heyn) i.e. turgidity and increased wall plasticity. Nevertheless, unless they are supplied with water they do not grow. It seems probable that the effect of higher wall plasticity, decreasing the turgor and hence increasing the suction force is in permitting the uptake of water and accompanying increase in area of the cell wall. Increases in elongation would be then due not directly to the increase in deformability of the cell wall but to the increase in suction force of the cell. As already mentioned, the work of Ursprung and Blum has shown that suction force is at a maximum in the most rapidly growing regions of roots.

It has been tacitly assumed that increases in irreversible deformability in reality are due to increases in plasticity. A supporter of "active growth" might object that these increases in deformability may be due to the rapid intussusception of wall particles during the action of growth substance ~~and that the increase in deform-~~

i.e. to a temporary excess of new wall over actual elongation and hence lowered turgor. It has not, in fact, been shown that growth substance does not effect the deposition of new wall material. Heyn (1931) originally thought that the fact that growth substance does not affect the fixation of elastic stretching indicated that it did not affect wall formation but he has since (1934) shown that the process of fixation is in all likelihood only a relaxation of elastically stretched layers.

e. Objects of the Present Work

The information concerning cell elongation and its connection with growth substance before the undertaking of the present work have been outlined. With respect to cell elongation itself it is clear that, between the two possible alternative mechanisms, plastic stretching or intussusception, a final decision has not as yet been possible. It is known, only, that growth substance brings about an increase in coleoptile deformability. Concerning the actual steps of growth substance action nothing has been learned. The first questions which it seems necessary to answer in the study of this problem are:

1) Does growth substance act as a true hormone, i.e. indirectly and in small amounts, or can it take part as a constituent, for example, in wall formation?

2) Does growth substance influence formation of cell wall or does it influence properties of the cell wall? In this way a final decision between intussusception of new material and plastic stretching may be obtained.

3) Does growth substance influence the wall directly or does it influence functions of the protoplasm?

4) If growth substance acts through functions of the protoplasm, what is the nature of the action?

These questions will be dealt with in the following sections.

PART III.

A GENERAL DISCUSSION OF THE MATERIALS AND METHODS

a. Plants

The *Avena* seedlings used in these experiments were of the pure line "Sieges Hafer" supplied by Dr. Åkermann of Svalöv. They were grown in the dark rooms of the plant physiology laboratory, at the humidity (85 to 90 per cent) shown by Went to be suitable for the carrying out of growth substance activity tests. The temperature was held constant to 0.2° during the course of an experiment but varied occasionally between 25° and 27° during the course of three years. Only red light was used in the carrying out of all of the experiments in order to avoid phototropic curvatures of the plants. Illuminating gas, minute traces of which causes great irregularities in the growth of the seedlings, was not present in the building.

The husked seeds were soaked in water for 4 or for 12 hours, germinated for 24 hours upon moist filter paper and then planted either in glass holders for tests (Went, 1928) or in a mixture of sand and leaf mold. At the total age of about 84 hours the coleoptiles were 2 to 3 cm. tall and ready for use.

b. Growth Substance

It has been shown by Nielsen (1930) that considerable amounts of growth substance are produced in cultures of the mold, *Rhizopus suinus*. The conditions for production of growth substance have been more thoroughly investigated by Bonner (1932) and by Thimann and Dolk (1933). The growth substance used in the present experiments

was obtained from the large scale culture of this organism, the preparation and purification being carried out by Dr. K. V. Thimann. The mold was grown upon a peptone-glucose culture medium and care was taken to keep the conditions aerobic, since this greatly increases the yield. At the end of three days the mycelium was filtered off, the medium concentrated under diminished pressure to 1 per cent of its volume, filtered, acidified, and extracted six to seven times with peroxide free ether. The bulk of the ether was distilled off, the residue taken up in a small volume of distilled water, boiled free of ether, and the solution chilled. Chilling causes the precipitation of a rather large quantity of oily material which was filtered off. The filtrate is a growth substance preparation of rather high purity which may be used directly for experiments. It has an activity of about 10^{-6} mgs. per plant unit (see below) as compared with cyrstalline growth substance which has an activity of about 1.3 10^{-8} mgs. per plant unit. Later in the course of the experiments as will be indicated preparations of higher activity were used. The author is greatly indebted to Dr. K. V. Thimann for these growth substance preparations.

c. Units

One growth substance unit, as used in the remainder of this paper, corresponds to that defined by Dolk and Thimann (1932). It is that amount of growth substance which when present in 1 cc. of solution will give after being mixed with 1 cc. of 3% agar and made into agar blocks of 10.7 mm^3, a curvature of 1° upon the standard decapitated *Avena* coleoptile under the standard conditions, of this laboratory. (Dolk and Thimann, 1932)

PART IV.

THE HORMONAL NATURE OF GROWTH SUBSTANCE

a. Introduction

The first question to be answered concerns the relation of the amount of growth substance in the plant to the amounts of the various substances undergoing transformation during elongation. In this part, it will be determined whether or not there is any simple stoichiometric relation between the number of molecules in a given amount of cell wall and the number of molecules of growth substance necessary to produce an equivalent amount of elongation. This determination involves the reasonable assumption that the new cell wall formed during the growth substance has the same constitution as that already deposited. This assumption is supported in Section ([illegible]) of this part. The experimental work is divided into five heads corresponding to the pieces of information necessary for the calculation of the desired relation. These heads are as follows:

1) The amount of growth substance entering the plant under the experimental conditions.
2) The amount of elongation brought about by a given amount of growth substance.
3) The actual increase in cell wall during a given amount of elongation.
4) The composition of the cell wall and hence the increase of each constituent during a given amount of elongation.
5) The increase in volume of cell wall during a given amount of elongation. This is an independent method of arriving at the result given by 3.

In addition the elongation produced by a given amount of growth substance under different experimental conditions will be determined for comparison.

b. Methods

Growth measurements. The growth measurements were carried out with an automatically controlled motion picture camera. The films, after development, were projected with a final enlargement of 100 times upon a screen, on which the images of the plants could be measured with considerable accuracy. The Leitz camera was operated with a "De Bouter intermittent Clinostat", which in turn was operated by an electric clock. A general description of this type of clinostat is given by F.A.F.C. Went (1929). The two plants which were photographed upon each film were placed about one meter from the camera with their roots in water. Beside each plant was placed a millimeter scale which was included in the photograph and from which the growth increments were measured.

Since the etiolated coleoptiles are very sensitive to light of wave length shorter than 5300 A°, the photographs were taken in orange light, using Wratten filter No. 24, which cuts off most of the light shorter than 5450 A°. Eastman supersensitive panchromatic film was used with an exposure of five seconds.

The experiments were carried out in a constant temperature room at 27°. The relative humidity was kept at 90%, with a variation of not more than 3-4%. Since the standard conditions for testing the activity tests of growth substance preparations by the curvature method used in this laboratory include a temperature of 25°, a second room was kept at this temperature and the activity tests carried out in it.

c. The Amount of Growth Substance Entering the Plant

It has recently been found (Thimann and Bonner, 1932) that the rate at which growth substance passes from solution in agar into the decapitated coleoptile is directly proportional to its concentration in the agar, and may be expressed by the equation

$$-\frac{dx}{dt} = k\frac{x}{v}$$

where x is the amount in a block of volume v. From experiments in which agar blocks containing growth substance after having produced curvatures were placed upon fresh decapitated coleoptiles, the rate constant of passage was determined. From the value of this constant the fraction of growth substance initially present which would pass out of blocks of different volumes was calculated, and shown to agree with the experimental results. For blocks of the standard size this fraction is 15%. It was also shown that for blocks of small volume a very large fraction of the growth substance initially present passes out, which accounts for the belief, held by earlier investigators, that all of the growth substance passes from block to coleoptile.

While the above work was concerned with blocks placed unilaterally upon coleoptiles, the present experiments are concerned with vertical growth in which blocks are placed flat upon the coleoptile stump. Since the area of contact is more than doubled, it might be expected that the proportion of growth substance passing into the plant in this case would also be more than doubled. For the evaluation of this proportion, large numbers of plants were decapitated and supplied with agar blocks containing a known amount of growth substance, precisely as was later done with the plants

whose growth rates were to be measured. At the expiration of 105 minutes, the blocks were removed, placed unilaterally upon decapitated test plants, and the resulting curvatures measured. A curvature θ is, as pointed out by Thimann and Bonner proportional to the concentration of growth substance in the agar block. Since blocks were made from a growth substance solution of known strength, they would, if placed upon test plants, give an initial curvature θ_o. From the curvature θ_1 given by the second test plant, the amount of growth substance in the block at the beginning of the second test is also known. Hence, the fraction of the initial quantity of growth substance which passed out of the block while it remained flat upon the coleoptile is given by $\frac{\theta_o - \theta_1}{\theta_o}$.

Table 1 gives a series of determinations of θ_1, and of this fraction, for different values of θ_o. The mean value of the fraction passing out is 34%.

(Insert Table 1)

d. The Amount of Growth Corresponding to a Given Amount of Growth Substance at 27°

Although it has been shown that the response of coleoptiles to unilateral application of growth substance is linear over a certain range, the response to symmetrical application has not been quantitatively followed. The response to different amounts of growth substance contained in agar blocks laid flat upon coleoptiles was therefore measured in order to determine whether this was also linear, and if so, over what range.

Preparation of plants. Coleoptiles exhibit their maximum rate of growth when they are about 3 cm. long (Konigsberger, 1922; see also below) and the rate of growth is not greatly different for plants a little shorter. Since for these experiments it is only necessary to choose plants of similar growth rates, straight plants of lengths between the above limits were chosen.

With the aid of a sharp blade the tip of the coleoptile was cut at a point 5-6 mm. from its apex, and the first leaf drawn out until only 2-3 mm. remained and cut off flush with the stump of the coleoptile. This piece of leaf was allowed to remain, in order to prevent the gelatin from flowing into the cylinder. The plants were then removed to the dark room containing the apparatus, and placed in position beside the millimeter scales. If the plants were to be supplied with growth substance this was done by fastening, with 15% gelatin, an agar block containing growth substance in the desired concentration upon the stump. The clinostat was next started and the first picture taken quickly as possible, which was in general 2-3 minutes after decapitation.

Two pictures, separated by a two minute interval, were taken each 15 minutes. The values for the growth increments in the subsequent tables are obtained from the means of these two pictures.

Rate of growth of decapitated coleoptiles. The rate of growth of decapitated coleoptiles, unsupplied with growth substance from an outside source, was first determined. Figure III gives graphically the results obtained from the mean values of 6 plants, the rate of growth in millimeters per 15 minutes being plotted against time. The rate of growth may be seen to decrease steadily after

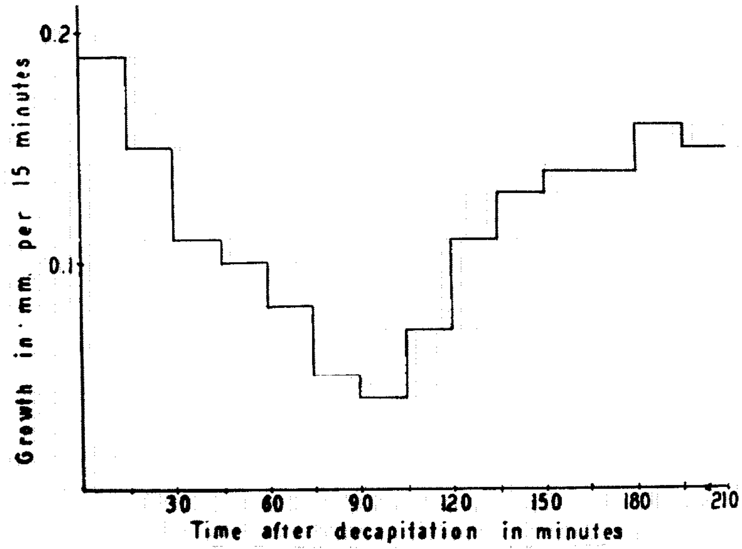

Figure 3. Growth Rate of Decapitated Coleoptiles not Supplied with Growth Substance.

decapitation, for 105 minutes. After this time the rate increases rather sharply, and reaches a constant level which is, however, not so high as the original. An inspection of the actual data (Table 2)

(Insert Table 2)

reveals that the mean values, plotted in Figure III, do not accurately represent the growth of the individual plants. In general, the plants tend to drop gradually to a minimum and mount quickly to the new constant level, but this is obscured by the mean, since the minima are somewhat spread out. This sudden physiological regeneration of a growth substance-producing region in the stump will be considered in the next section. It is also an indication that growth substance production by zones of the coleoptile below the tip cannot take place until a low concentration is reached.

The growth of Avena coleoptiles after decapitation has been previously studied by Dolk (1926) and by Heyn (1931). These investigators found exactly the same type of response as in the present case, but since they worked at lower temperatures (21° and 23° respectively) they found the minimum to occur somewhat later. As mentioned before, the appearance of new growth after the minimum is due to the production of growth substance by the uppermost region of the coleoptile stump, which previously did not produce growth substance. The growth of a decapitated coleoptile before the minimum represents, then, the growth due to whatever growth substance was left in the plant after removal of the tip.

Rate of growth of plants supplied with low concentrations of growth substance. In the next series of experiments, plants were supplied with agar blocks containing 9.9 and then 14.5 "plant units"

of growth substance. Now, the rate of passage of growth substance from block to plant depends upon the concentration of growth substance in the block. Hence, even by allowing the block to remain for a very long time upon a coleoptile, the complete transference of the growth substance to the plant would be impossible. It was found much more convenient to remove the block after a definite time and to determine, as in the previous section, the amount of growth substance which had actually entered the plant. For comparison with plain decapitated coleoptiles, the blocks were allowed to remain 105 minutes, that is, until physiological regeneration would have occurred if the plants had not been supplied with growth substance.

The mean growth rates of these plants are shown in Figure IV, in which the curve for decapitated coleoptiles without growth substance is inserted for comparison. The data are included in Table 3. The 9.9

(Insert Table 3)

unit curve is somewhat irregular, but falls, in general, although more slowly than that of plain decapitated coleoptiles, to a rather low value after 3 hours without exhibiting any minimum. The 14.5 unit curve, is, until removal of the block, nearly constant at a value slightly higher than that of the initial growth rate of plain decapitated coleoptiles. After removal of the block the rate falls through a minimum and rises to a constant value. The minimum is not so low and is reached one half hour earlier than the minimum of plain decapitated coleoptiles. The shape of the curve resembles greatly, however, that of Figure III.

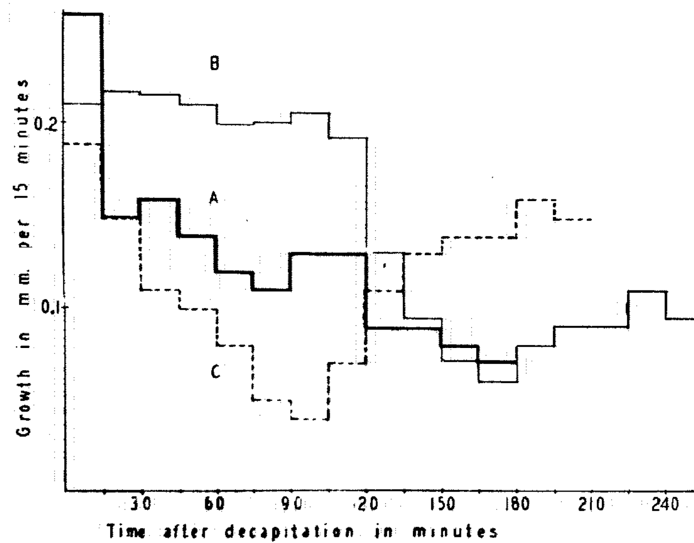

Figure 4. Growth Rates of Coleoptiles Supplied with Small Quantities of Growth Substance. Curve A, 9.9 units of Growth Substance; Curve B, 14.5 units of Growth Substance; Curve C, no Growth Substance.

The coleoptile recommences the production of growth substance in this case, when it has reached the minimum growth rate, which is after 3 hours. The total growth between decapitation and this minimum is due (a) to the added growth substance (i.e. given up by the block), (b) to the residual growth substance left in the plant after decapitation. It is clear that the growth due only to the added growth substance is the total growth from the time of decapitation to the minimum, less the amount of growth of plain decapitated coleoptiles to their minimum. If it be assumed, as seems justified, that at the end of 3 hours the 9.9 unit curve also begins to mount toward a new constant level, the growth due to the 9.9 unit block may be determined in the same way.

Growth rates of plants supplied with higher concentrations of growth substance. The rates of growth due to successively higher concentrations of growth substance was next studied, and Table 3 gives the means of the determinations at each concentration. The growth rates of four of the concentrations are plotted in Figure V. In each case, except one at very high concentration, the addition of more growth substance to the plant results in an increase of growth. In general, also, the response to a large concentration of growth substance is immediate, that is, even in the first period the growth is larger than that of plain decapitated coleoptiles. After removal of the block the curves drop to a low constant value which is approximately the same in each case. The time between the removal of the block and the attainment of this low value is, however, longer the higher the concentration. The fact that this final constant

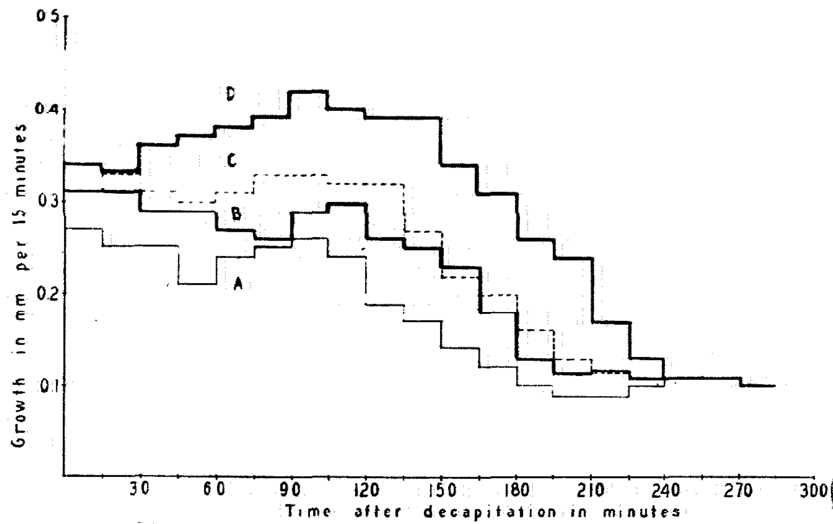

Figure 5. Growth Rates of Decapitated Coleoptiles Supplied with Large Quantities of Growth Substance. Curve A, 19.3 Units of Growth Substance; Curve B, 29.0 Units of Growth Substance; Curve C, 39.5 Units of Growth Substance; Curve D, 116.0 Units of Growth Substance.

level is lower for plants supplied with growth substance than for the plants of Figure III may be attributed to the greater utilisation of other necessities of growth in the presence of more growth substance.

None of the curves of Figure V exhibit a clear minimum. Heyn has stated that the removal of the block is exactly similar to removal of the original tip, and that the production of new growth substance commences at a definite time later. If this were true, no minima would be expected with the higher concentrations of growth substance, since the growth substance left after the removal of the block would give rise to a rather large amount of growth. This growth would obscure the minimum. The quantitative relation between growth and growth substance which may be obtained from the present data with the aid of a different assumption, however, lead to the belief that Heyn's interpretation is not the correct one, and that the true reasons for the absence of minima are those which will be brought forward below.

<u>The linear relationship between growth and growth substance.</u> The zones which produce growth substance after physiological regeneration do not in the presence of a tip, produce any appreciable amount. This suggests that the growth substance has, as a secondary function, the inhibition, by its presence in sufficiently high concentrations of the production of growth substance by the lower zones. In a plain decapitated coleoptile or in one supplied with a small amount of growth substance, removal of the tip, or of the agar block, is followed by transport of all of the small amount of residual growth substance from the top

zones to the lower (Van der Wey, 1932). The result is that when the concentration of growth substance in the top of the stump has become low enough to permit regeneration, the growth rate of the coleoptile as a whole has become very small, and the growth curve therefore passes through a minimum. If, however, a decapitated coleoptile is supplied with a large amount of growth substance, a different result would be expected. After removal of the block, the growth substance which is left in the upper zones is transported to the lower, but these zones are unable to utilise such a large amount as quickly as it is brought in. The result is that while the concentration in the uppermost zone falls to a value sufficiently low to permit regeneration, the concentration in the lower zones remains high, they grow continuously, and no minimum in the total growth rate is apparent. The essential difference between the situation of plants supplied with large and with small amounts of growth substance is, then, one of distribution.

In accordance with this view there is, for plants supplied with large concentrations of growth substance, no exact time at which growth due to the growth substance from the block ceases, and that due to growth substance from the regenerated tip commences. As an approximation it can be assumed that the time at which the growth reaches its final low constant level is the time at which the growth substance from the block is used up. The correction is in any event small.

The total growth of these plants from time of decapitation to establishment of the final level, less that due to the residual growth substance, represents, then, in each case the growth due to

the amount of growth substance which has entered the plant, and this amount is, as already pointed out, proportional to the concentration applied.

Table 4 gives the growth thus obtained from each concentration of growth substance. From the total growth, from the decapitation

(Insert Table 4)

to the final level, was subtracted the total growth, to their minimum, of the decapitated coleoptiles of Figure III. For comparison of the growth per unit of growth substance, this value is in each case also given. For the 4 lowest concentrations this ratio, is within a few per cent, constant. Above 29 units the ratio decreases somewhat, that is, a further addition of growth substance produces proportionately less growth. Above 58 units the ratio decreases sharply; in fact 116 units gives but little more growth. Figure VI summarizes the amounts of growth from Table 4, plotted against growth substance entering the plant, and shows that the growth response of coleoptiles to growth substance is, up to 10 units (i.e. 29 units applied) linear, while above 20 units (i.e. 58 units applied) more growth substance, on the average, produces little increase in growth. For comparison the curvatures produced by unilateral growth are also plotted against amount of growth substance entering the plant (dotted line). The limiting amounts can be seen to be much higher for straight growth than for curved growth. This is probably due principally, as pointed out by Du Buy and Neurnbergk (1932), to a relatively slow non polar transport of growth substance, particularly at the cut surface.

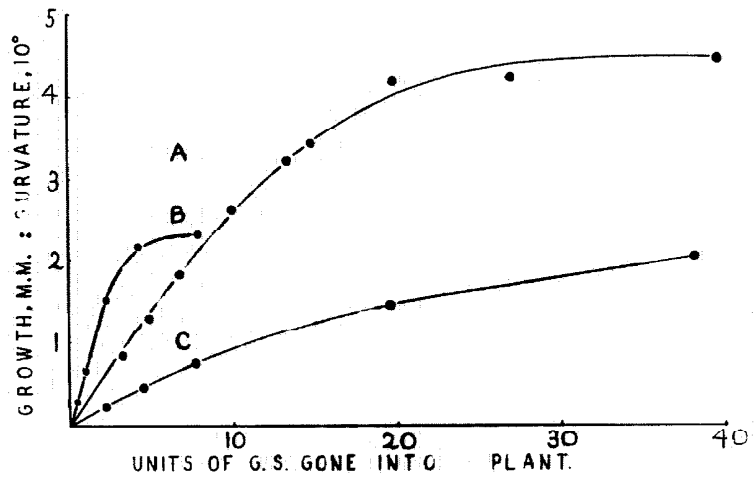

Figure 6.Amount of Growth Resulting from the Entrance of Different Amounts of Growth Substance into the Plant. Curve A, Vertical Growth at 27 C.Curve B, Curved Growth at 25 C.Curve C, Vertical Growth at 15 C.

e. The Amount of Growth Corresponding to a given Amount of Growth Substance at 15° C.

It is of interest to know whether under different conditions one molecule of growth substance brings about the same amount of growth. Accordingly a complete set of measurements analogous to the preceding was carried out at 12° lower temperature, i.e. at 15°. For this purpose a thermostat having a central chamber surrounded by a water bath was constructed. The central chamber, made of copper for efficient heat transfer, was equipped with double glass doors through which the plants were photographed without difficulty. Humidity of the inner chamber was controlled by the presence of solid KCl, which kept the humidity at about 80%. The outer chamber, filled with water, was cooled by ice and regulated thermostatically to within 0.2° of the desired temperature. A thermometer in the inner chamber showed that within 2 to 3 minutes after closing of the double glass doors the chamber took on the temperature of the outer bath.

Table 5 gives the results of measurements analogous to those recorded in Table 3. At fifteen degrees the minimum is reached by decapitated plants in two hours instead of in 105 minutes as at 27°, and in the subsequent three hours no regeneration is to be found. This is in agreement with the work of Tsi Tung Li (1930) who found that regeneration is delayed at lower temperatures. It also simplifies the calculation of elongation per unit growth substance since the only correction is that for the residual growth substance. Table 6

(Insert Tables 5, 6, & 7)

summarizes the growth per unit of applied growth substance as did Table 4. The amount of growth substance passing into the plant was

next determined in the same way as was done for 27°. The tests for growth substance left in the blocks were, of course, carried out at 25°. Table 7 shows that the fraction passing out of the block at 15° in 2 hours is even slightly larger than that passing out at 27° in 105 minutes, i.e. the uptake of growth substance by the plant is practically independent of the temperature. The significance of this fact is not clear since as found by Van der Wey (1932), the transport of growth substance has a relatively high temperature coefficient. It is possible that it is due to limitation of rate of entry of growth substance by the rate of passage across the block-plant surface, a diffusion process of low temperature coefficient. In Figure VI . is plotted the elongation per unit of growth substance entering the plant at 15° as well as at 27°. It may be seen that the amount of elongation per molecule of growth substance is more than twice as great at 27° as at 15°.

f. The Composition of the Cell Wall

In order to convert growth, in terms of coleoptile length, into amounts of material actually laid down, the relation between coleoptile length and amount of cell wall material must be known.

The material used in these experiments consisted of coleoptile cylinders, with tips removed, either of young plants of the size used in the previous work, or of old plants from which the third leaves would soon break. It was found that altho the weight of "cell-walls" per mm. was greater in the older plants, yet the composition of the cell walls was the same. The old plants were more convenient to use, since they provided much more material with which to work.

Table 8 gives the dry weights per mm. of a number of samples of young coleoptiles. The weights depend considerably upon the conditions of drying, since although samples dried at the same time (100° for 24 hours) check fairly well, there is more variation between samples dried at different times. Nevertheless, the dry weight per mm. is relatively constant. This constancy of dry weight per mm. might have been expected, since the coleoptile is, except for the tip, of a very nearly uniform diameter, which varies but little from plant to plant. The dry weight per mm. of coleoptiles grown at 15° was found to be practically the same as that of coleoptiles grown at 25°.

The next step was to obtain cell walls free of cytoplasm and cell sap. In order to accomplish this, samples were thoroughly ground, and extracted several times with cold water. This was followed by two hours of extraction with repeated portions of hot water. The residue was retained upon a coarse filter, through which the colloidal solution obtained from the washings readily passed.

This fraction which is not dissolved or suspended in water, can be considered as the cell wall. It was dried to constant weight at 95°. Table 9 gives the amounts of this fraction obtained from several samples of young coleoptiles.

(Insert Tables 8 & 9)

Extraction of the cell wall fraction for several hours with ether gave no decrease in dry weight, and it was therefore concluded that no appreciable amount of fatty substances were present. The pectic substances were extracted by the method of Nanji and Norman (1928) in which the cell wall samples are heated at 80° for 24 hours with 0.5% ammonium oxalate. Results for two such extractions are

included in Table 10.

(Insert Table 10)

The amount of cellulose present in material of this kind may be determined approximately by the "crude fiber". The samples were heated successively with 2% H_2SO_4, with 2% KOH and with alcohol, and the residue dried and weighed. Pentosans may remain to a small extent as impurities, as has been shown by Lüdtke (1931). For unlignified tissues, however, the crude fiber is principally cellulose. The degradation was also carried out in steps upon young and old coleoptiles, in order to compare the composition of the two. These results are included in Table 10. It may be seen that the amount of substance removed at each step does not depend upon the age of the coleoptile. Since this is the case, the ratio of the water-insoluble fraction to the crude fiber was determined several times upon old coleoptiles (see Table 10).

Determinations of the nitrogen in the samples were carried out by the micro-Kjeldahl method. An approximation to the per cent of protein present in the wall may be obtained by multiplying the per cent of nitrogen by 6.2. The nitrogen found in two samples of cell walls, together with the calculated protein, is included in Table 10.

A summary of the results is given in Table 11, in which the different fractions of the cell wall are expressed as percentage

(Insert Table 11)

of the total average dry weight. It might seem at first sight rather surprising that only about 12% of the coleoptile may be considered as consisting of cellulose, but that such large amounts of hemicellulose

and proteins are present in the cell wall. Link (1929) has analysed Zea seedlings, of an age comparable to that of the Avena plants used here, and found that only 14-18% of their total dry weight is pure cellulose, and that 10-12% is pure xylan.

It will be assumed that the increment of length produced by the action of growth substance is composed of normal tissue, that is, that the processes of cell wall formation keep up with the processes of cell-wall elongation, irrespective of whether these two sets of processes are connected or not. That this is actually the case is indicated by the following experiment: coleoptiles were supplied every 2 hours for 14 hours with fresh agar blocks containing 100 units of growth substance. At the end of this time the coleoptiles were cut off, dried, and weighed, together with normal control coleoptiles. The results were as follows: The plants supplied with growth substance increased in length an average of 1.9 cm. (12 plants) while normal coleoptiles in the same time increased by 1.0 cm. (6 plants). The dry weight of the coleoptiles supplied by the growth substance was $5.2 \cdot 10^{-2}$ mg. per mm., while that of normal coleoptiles carried out at the same time was, in two samples, 5.1 and $5.2 \cdot 10^{-2}$ mg per mm.

g. The Volume of the Cell Walls

A determination was made of the volume of new cell walls which appear as the result of the action of a known amount of growth substance. Since the estimation of the area of the cell wall is at best rather uncertain, the coleoptile will for the present purposes be considered as a hollow cylinder. This simplifies the calculations without greatly affecting the result. In the case of longitudinal

growth, only the longitudinal walls need be considered.

The inside and outside diameters, as well as the diameters of the individual cells of a large number of coleoptiles were measured under a microscope with a calibrated eye-piece micrometer. The number of cell layers were counted and found to be fairly constant. Table 12

(Insert Table 12)

gives the mean values of these measurements. Figure VIII shows a section, drawn to scale, through a diagrammatic coleoptile of the dimensions given in Table 12. The circumference of each row of walls may be easily found. The mean circumference, when divided by the diameter of the cells occupying this particular row, gives the number of cells of this kind present, and hence the number of radial cell-walls. The total length of cell walls found in this way, gives, when multiplied by 1 mm., the area of cell wall in 1 mm of coleoptile. These calculations are given im Table 13. The value obtained for

(Insert Table 13)

the total wall area is clearly a minimum figure, since it will be increased by (a) irregularities in the shapes of the cells (b) inter-cellular spaces, and (c) the vascular bundles, which are made up of smaller cells.

Measurements of the cell wall thickness gave a mean value of 0.4μ, the outermost layer of cuticularized walls not being included. From these values for area and thickness, the volume of the cell wall will be calculated.

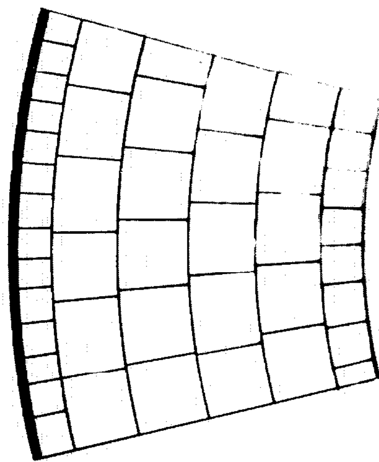

Figure 7. Portion of a Cross Section through a Diagramatic Coleoptile.

h. Ratio of Cell Wall Constituents produced to Growth Substance used

From Figure VI, it is clear that at 27° the response of the coleoptile is linear for quantities of applied growth substance smaller than about 35 units. We will therefore consider the response to 29.0 units, which is well within this range.

Table 4 shows that the addition of 29 units produces a growth of 2.66 mm. From analysis of the coleoptile, (Table 9) it was found that the weight of cell walls in 1 mm. is 1.64×10^{-2} mg. Consider for the moment that only the actual cellulose of the cell-wall hasbeen laid down by the action of growth substance, if such a reaction, in fact, takes place. This assumption is to some extent justified by the consideration that the principal skeletal material of the coleoptile is certainly the crystallised cellulose. It is clear that this procedure will lead to a minimum value for the carbohydrate deposited as a result of growth substance action. Table 11 has shown that the cellulose (crude fiber) constitutes 42% of the cell wall, and hence the amount of cellulose deposited as a result of 29 units is

$$2.66 \times 1.64 \times 10^{-2} \times 0.42 = 1.83 \times 10^{-2} \text{ mg.}$$

Now cellulose is made up of glucose residues ($C_6H_{10}O_5$) of molecular weight 162, and 1 millimole contains 6.06×10^{20} such residues. Therefore, the number of such glucose residues laid down as cellulose under the action of 29 units is

$$1.83 \times 10^{-2} \times \frac{1}{162} \times 6.06 \times 10^{20} = 6.84 \times 10^{16} \text{ residues.}$$

In order to arrive at a figure for the amount of growth substance involved, it is necessary to use the value given by Kögl and Haagen-Smit (1931), since, while their substance is not necessarily identical with that used here, it is closely similar in action, and was obtained by them in the crystalline state. According to them, one unit is equal to 1/30,000,000 mg. The unit which they use is defined as giving a 10° curvature, whereas ours is defined as giving a curvature of 1°. The blocks which they use have a volume of 2 mm^3 and hence by the procedure of Table 4 of Thimann and Bonner (1932), 58% of the growth substance passes out of the block during their test. The amount passing out of the block in our test is 15%, and hence we need to have 4 times their amount in our block to give the same curvature. Our unit is then

$$4 \times 3.3 \times 10^{-8} \times 0.1 = 1.3 \times 10^{-8} \text{ mg.}$$

In this experiment $29 \times 1.3 \times 10^{-8} = 3.8 \times 10^{-7}$ mg. were used.

Table 1 shows that when blocks are placed flat upon coleoptiles, as in this experiment, 34% of the growth substance present in the block passes into the plant, that is, in this case,

$$0.34 \times 3.8 \times 10^{-7} = 1.3 \times 10^{-7} \text{ mg.}$$

pass into the plant. The mean molecular weight of the growth substance obtained by Kögl and Haagen-Smit is 328, and this figure is checked by the value obtained in this laboratory. The number of molecules of growth substance entering the plant is then

$$6.06 \times 10^{20} \times 1.3 \times 10^{-7} \times \frac{1}{328} = 2.40 \times 10^{11} \text{ molecules.}$$

We have, then, from the above, 2.40×10^{11} molecules of growth substance for 6.8×10^{16} molecules of $C_6H_{10}O_5$, that is, one molecule of

growth substance has acted in the laying down of 3×10^5 $C_6H_{10}O_5$ residues. Since this ratio is greatly different from 1, it is at once clear that <u>the growth substance is not involved directly in the deposition either of cellulose, or of total cell-wall carbohydrate</u>.

We will now compare the number of molecules of growth substance with the number of micelles of cellulose deposited. As Katz (1928) points out, the micelles of fibers, since they are not visible even with ultraviolet light, must be shorter than 500 A°. Katz gives the most probable size of the micelles as 60 - 110 A°. The unit cell of cellulose is 8.7 x 7.9 x 10.3 A°, and contains four glucose residues (Meyer and Mark, 1928). If we take the micelles as 87 x 40 x 103 A°, a figure which accords with that of Katz, they each contain 2000 glucose residues. Since 1 growth substance molecule acts in the deposition of 3×10^5 glucose residues, it acts, therefore, in the deposition of

$$3 \times 10^5 \times \frac{1}{2000} \text{ or } 140 \text{ micelles.}$$

Since there is no reason to believe that the micelles of cellulose in young tissues, such as coleoptiles, are any larger than those of the cotton and ramie fibers, with which the micelle size determinations were made, it is clear that <u>there cannot be any one to one correspondence between growth substance molecules and micelles formed in growth</u>.

A second, less reliable, method for determining the number of molecules of cellulose laid down by one molecule of growth substance is as follows:

It has been found that the total area of longitudinal cell walls per mm. of coleoptile length was 40.3 $mm.^2$ and the mean wall

thickness was 0.4 μ. Hence the volume of the cell walls is

$$40.3 \times 4 \times 10^{-4} = 1.61 \times 10^{-2} \text{ mm}^3$$

As above, the unit of cellulose structure is the block containing four glucose residues, and having dimensions as follows:

$$8.7 \times 7.9 \times 10.3 \text{ A}^\circ\text{, or a volume of } 7.08 \times 10^{-19} \text{ mm}^3.$$

The specific gravity of cellulose will not be greatly different from that of the other cell wall material, and we can assume the cell wall material to consist, therefore, of 42% cellulose by volume. Hence 1 mm. of coleoptile contains

$$1.61 \times 10^{-2} \times \frac{1}{7.08} \times 10^{-19} \times 0.42 = 0.96 \times 10^{16} \text{ unit blocks.}$$

Since each block contains four hexose residues, there are 3.82×10^{16} hexose residues per mm. of coleoptile.

An increase in length of 2.66 mm., the result of the application of 29 units of growth substance, therefore involves the production of $3.82 \times 10^{16} \times 2.66$ or 10.2×10^{16} new hexose residues. Since no allowance has been made for the volume taken up by water in the wall, this figure may be somewhat too high. Considering the approximate nature of the determination of the cell wall volume, the agreement between this figure and that obtained above from the weight of the wall must be regarded as satisfactory. Using the above figure, we find that one molecule of growth substance leads to the deposition of 4.6×10^5 hexose residues, instead of 3×10^5 as in the first calculation.

Since from the above it is scarcely possible to assign a stoichiometrical relationship between the growth substance and the materials of the cell wall, another possibility was investigated,

namely that the growth substance is distributed in a monomolecular layer over the surface of the cell, and is thus by some means able to promote the elongation of the cell wall area which it covers.

According to the analyses of Kögl and Haagen-Smit, the growth substance has the approximate formula $C_{18}H_{32}O_5$. Since it is also known to be an unsaturated acid (Kögl, Erxleben, and Haagen Smit, 1933) one may apply Langmuir's (1917) measurements on surface films of the fatty acids, from which an unsaturated C_{18} acid will have a maximum area of 4.6×10^{-13} mm^2. As stated above, the cell wall has an area of 40.3 mm^2 per mm. of coleoptile, and hence 2.66 mm. have an area of 107 mm^2. The number of molecules of growth substance involved is 2.3×10^{11}, which from the above value, can only cover a maximum area of 0.11 mm^2. Therefore, to form a monomolecular layer only on the surface of the new cell wall laid down, each molecule of growth substance would have to cover approximately 1000times its own maximum area. In order to cover the surface of all the growing cells it would have to cover even more. Now, for the growth substance to act by producing any change in permeability, it must, presumably, produce a change in surface activity; to do this it must form at least a monomolecular layer upon the cell wall, which is clearly impossible. Furthermore the measurements of Rideal (1925) and of Langmuir (1927) show that the rate of passage of water from a liquid surface into the vapor is greatly decreased by the presence of surface films of fatty acids. Thus it is likely that any effect of growth substance upon permeability to water would be in the direction of decreasing rather than increasing it. <u>The theory that the action of growth</u>

substance is directly one of permeability change is thus given the appearance of being extremely improbable.

The results of the present section may be summarized as follows:

1) One growth substance molecule, at 27°, brings about elongation accompanied by the following changes

a) molecules of glucose to cellulose	$3x10^5$
b) micelles of cellulose formed	$1.4x10^2$
c) molecules of pectin formed	$7x10^3$
d) molecules of protein (in the cell wall) formed, mol. wt. 35000	$3.9x10^2$
e) molecules of carbohydrate converted to hemicellulose	$1.6x10^5$
f) molecules of pentose converted into pentosans	$8.7x10^4$.

Since there is so little growth substance in comparison with the structural material it seems certain that growth substance is a true hormone and not itself a structural material. There is, moreover, far too little growth substance present to react stoichiometrically with any of the constituents of the cell wall and thus alter its properties. The theory of VanOverbeek (1933) that growth substance by adsorption on the micelles of cellulose alters the interfacial tension between them and the intermicellar fluid is out of the question.

2) There is insufficient growth substance to form a monomolecular layer on the protoplasmic membranes. Moreover, growth substance should decrease rather than increase the water permeability of surfaces on which it is adsorbed. It is therefore not likely that growth substance effects growth through an increase of water permeability.

3) One growth substance molecule causes less than one half as much elongation at 15° as at 27°, altho the amount of coleoptile formed per unit of elongation is the same. It is therefore impossible that one growth substance molecule causes the intussusception of a given amount of wall material.

TABLE 1.

PERCENT OF GROWTH SUBSTANCE PASSING INTO PLANT
FROM BLOCKS FLAT UPON THE COLEOPTILE STUMP

Units applied (Initial possible curvature) θ_0	Units remaining (Curvature on 2nd application) θ_1	% passed out of block $\frac{\theta_0 - \theta_1}{\theta_0} x100$	Number of Plants
14.4	9.0	37.5	17
14.4	9.8	32.0	15
19.2	13.0	32.3	21
19.2	12.0	37.5	16
	Mean % passed out of block	34	

TABLE 2

GROWTH RATES OF DECAPITATED COLEOPTILES UNSUPPLIED WITH GROWTH SUBSTANCE

Growth increments mm/15 min.

Plant No.	Time after decapitation in minutes													
	15	30	45	60	75	90	105	120	135	150	165	180	195	210
II-1	0.12	0.21	0.10	0.14	0.07	0.09	0.01	0.15	0.20	0.19	0.19	0.20	0.10	—
II-2	—	0.09	0.09	0.09	0.08	0.02	0.08	0.16	0.12	0.10	0.11	0.15	—	—
IV-1	—	0.12	0.04	0.04	0.03	0.03	0.04	0.00	0.01	0.11	0.16	—	—	—
IV-2	—	0.11	0.05	0.10	0.06	0.02	0.03	0.04	0.15	0.13	0.10	—	—	—
VI-1	0.23	0.23	0.22	0.09	0.13	0.04	0.06	0.04	0.02	0.10	0.10	—	—	—
VIII-1	0.20	0.12	0.13	0.17	0.11	0.14	0.04	0.03	0.09	0.09	0.16	0.07	0.16	0.15
Means	0.19	0.15	0.11	0.10	0.08	0.05	0.04	0.07	0.11	0.13	0.14	0.14	0.16	0.15

TABLE 3

AVERAGE GROWTH RATES OF COLEOPTILES UNDER THE INFLUENCE OF DIFFERENT AMOUNTS OF GROWTH SUBSTANCE

27° C

Growth increments per 15 minutes

Amount of g.s. in block.	Time after decapitation in minutes																		
	15	30	45	60	75	90	105	120	135	150	165	180	195	210	225	240	255	270	285
0	0.19	0.15	0.11	0.10	0.08	0.05	0.04	0.07	0.11	0.13	0.14	0.14	0.16	0.15					
9.9	0.26	0.15	0.16	0.14	0.12	0.11	0.13	0.13	0.09	0.09	0.08	0.07							
14.5	0.21	0.22	0.22	0.21	0.20	0.20	0.21	0.19	0.13	0.01	0.07	0.06	0.08	0.09	0.09	0.11	0.10		
19.3	0.27	0.35	0.25	0.21	0.24	0.25	0.26	0.23	0.19	0.17	0.14	0.12	0.10	0.09	0.09	0.10	0.11		
29.0	0.31	0.31	0.29	0.29	0.27	0.26	0.29	0.30	0.26	0.25	0.23	0.18	0.13	0.11	0.12	0.11	0.11	0.11	
39.5	0.31	0.33	0.31	0.30	0.31	0.33	0.33	0.32	0.32	0.27	0.22	0.20	0.16	0.13	0.11				
43.5	0.28	0.28	0.28	0.30	0.30	0.32	0.34	0.31	0.36	0.34	0.28	0.25	0.19	0.15	0.12	0.12	0.12		
58	0.35	0.32	0.35	0.36	0.37	0.40	0.42	0.37	0.42	0.42	0.33	0.30	0.23	0.20	0.14	0.12	0.12	0.13	
79	0.33	0.41	0.33	0.37	0.36	0.36	0.47	0.45	0.32	0.35	0.28	0.31	0.31	0.23	0.15	0.11	0.09		
116	0.34	0.33	0.36	0.37	0.38	0.39	0.42	0.40	0.39	0.39	0.39	0.31	0.26	0.24	0.17	0.13	0.11	0.10	0.10

TABLE 4.

TOTAL GROWTH AND ADDITIONAL GROWTH RESULTING FROM APPLICATION OF GROWTH SUBSTANCE FOR 105 MINUTES

Units applied	Number of plants	Total growth (see text) mm.	Time (see text) min.	Additional growth (see text) mm.	Growth / Units applied
0	6	0.71	105	0	
9.9	3	1.54	180	0.83	0.084
14.5	10	2.03	180	1.32	0.091
19.3	7	2.57	180	1.86	0.096
29.0	7	3.37	195	2.66	0.092
39.5	6	3.91	217	3.20	0.081
43.5	7	4.05	217	3.34	0.076
58.0	6	4.98	225	4.27	0.074
79.0	4	4.96	225	4.25	0.054
116.0	6	5.22	240	4.51	0.039

TABLE 5

AVERAGE GROWTH RATES OF COLEOPTILES UNDER THE INFLUENCE OF DIFFERENT AMOUNTS OF GROWTH SUBSTANCE

15°C

Time after decapitation in minutes

Growth increments mm/15 min.

Amount of g.s. in block	No. of plants	15	30	45	60	75	90	105	120	135	150	165	180	195	210	225	240
0.0	9	0.16	0.17	0.16	0.14	0.11	0.09	0.07	0.04	0.03	0.03	0.03	0.03	0.02	0.02	0.02	0.02
6.1	9	0.21	0.20	0.17	0.13	0.10	0.09	0.08	0.07	0.06	0.04	0.03	0.03	0.02	0.02	0.02	0.02
12.5	8	0.21	0.20	0.18	0.16	0.15	0.12	0.10	0.08	0.07	0.06	0.04	0.03	0.02	0.02	0.02	0.02
20.9	9	0.17	0.18	0.17	0.16	0.14	0.13	0.11	0.11	0.12	0.12	0.11	0.10	0.07	0.08	0.06	0.03
54	7	0.19	0.19	0.16	0.16	0.17	0.16	0.15	0.15	0.15	0.13	0.13	0.13	0.11	0.09	0.06	0.06
108	5	0.17	0.18	0.17	0.19	0.21	0.20	0.23	0.21	0.20	0.17	0.15	0.15	0.15	0.14	0.13	0.10

Amount of g.s. in block	No. of plants	255	270	285	300	315	330	345	360	375	390
0.0	9	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.02
6.1	9	0.02	0.01	0.02	0.02	0.02	—	—	—	—	—
12.5	8	0.02	0.02	0.02	0.02	0.02	0.02	—	—	—	—
20.9	9	0.03	0.02	0.02	0.02	0.02	0.02	—	—	—	—
54	7	0.06	0.05	0.05	0.04	0.02	0.02	0.02	0.02	0.03	—
108	5	0.07	0.06	0.07	0.05	0.03	0.03	0.03	0.03	—	—

TABLE 6.

TOTAL GROWTH AND ADDITIONAL GROWTH RESULTING FROM APPLICATION OF GROWTH SUBSTANCE FOR 120 MINUTES. 15°C.

Units Applied	No. of plants	Total growth mm.	Time min.	Additional growth mm.	Growth / Applied g.s.
0	9	0.94	120	—	—
6.1	9	1.18	150	0.24	0.039
12.5	8	1.40	180	0.46	0.037
20.9	9	1.75	225	0.81	0.039
54	7	2.39	300	1.45	0.027
108	5	3.03	315	2.09	0.019

TABLE 7.

PERCENTAGE OF GROWTH SUBSTANCE PASSING INTO PLANT FROM BLOCKS FLAT UPON COLEOPTILE STUMPS. 15° C.

Test	Units applied	Units remaining	Units passed out of block	% passed out of block	Plants
1	12.7	7.9	4.8	37.9	12
2	12.7	8.3	4.4	34.6	12
3	12.7	8.5	4.2	33.0	12
4	12.7	7.8	4.9	38.7	12
5	12.7	8.0	4.7	37.0	12
		Means	4.6	36.2	

TABLE 8.

DRY WEIGHTS OF COLEOPTILES

Aggregate length of coleoptiles in mm.	Weight in mg.	Weight per mm., grams$\times 10^{-5}$
332	16.9	5.09
348	18.0	5.17
341	18.6	5.46
349	19.2	5.51
408	25.8	6.32
409	26.0	6.35
207	12.3	5.95
132	8.3	6.30
173	10.5	6.08
179	11.6	6.50
336	17.6	5.25
389	17.0	4.25
643	33.9	5.23
200	10.7	5.35
306	17.8	5.81
300	17.1	5.70
	Mean	5.81

TABLE 9.

WEIGHT OF CELL WALLS FROM YOUNG COLEOPTILES

Aggregate length of coleoptiles in mm.	Weight of cell wall in mg.	Weight of cell wall per mm. in grams x 10^{-5}
498	8.10	1.63
635	11.4	1.79
687	10.9	1.50
745	12.8	1.72
731	11.3	1.55
637	10.5	1.65
	Mean	1.64

TABLE 10

ANALYSIS OF CELL WALLS OF COLEOPTILES

Weight of cell walls	Weight after extraction with oxalate	Weight after extraction with 2% H_2SO_4	Weight after extraction with 2% NaOH	Total N	Protein
22.3	20.4	11.4	8.6	---	---
47.6	43.9	25.0	17.4	---	---
21.0			9.7	---	---
25.2			11.1	---	---
22.8			10.3	---	---
	14.4	--	--	0.322	1.93
	19.5	--	--	0.392	2.08

TABLE 11

SUMMARY OF COMPOSITION OF COLEOPTILE CELL WALL

Fraction	% of cell wall	% total coleoptile
Removed by oxalate (pectic substances)	8	2.3
Removed by dilute acid less protein (hemicellulose)	28	8.2
Removed by dilute alkali (further pentosans)	10	2.8
Protein	12	3.1
Residue (cellulose)	42	11.9
	100	28.3

TABLE 12

MEAN VALUES OF DIAMETERS AND CELL SIZE OF COLEOPTILES

Outside diameter	1.18 mm.
Inside diameter	0.76 mm.
Number of cell rows	6
Diameter of epidermal cells	0.021 mm.
Diameter of sub-epidermal cells	0.024 mm.
Diameter of parenchyma cells	0.045 mm.
Diameter of inner dermal cells	0.024 mm.

TABLE 13

CALCULATION OF THE CELL WALL AREA PER MM. OF COLEOPTILE

Cell row	Diameter of wall.	Circumference mm.	No. of cells in row (circ./diam. of cells)	No. of cells x length of cell wall
Epidermis outside wall	1.18	3.71		
			173	3.63
Epidermis inside	1.14	3.58		
			146	3.50
Sub-epidermal parenchyma	1.09	3.42		
			73	3.28
Parenchyma 1	1.00	3.14		
			67	3.02
2	0.91	2.86		
			60	2.70
3	0.82	2.58		
			103	2.47
Inner dermal layer	0.76	2.39		
		21.68		18.62

Total area per mm. of coleoptile = 21.68 + 18.62 = 20.28 mm^2.

PART V.

THE RELATION OF WALL FORMATION TO CELL ELONGATION

a. Introduction

In the general introduction it was pointed out that active wall growth by intussusception has been advocated since the time of Krabbe and that fixation of elastic stretching by intussusception forms an integral part of the theory of Sachs. Söding and Strugger among the present investigators of cell elongation advocate intussusception, altho in neither case has any special evidence concerning this process been put forth. In Part IV it was shown that one growth substance molecule does not bring about the formation of any given amount of cell wall. More complicated actions of growth substance upon intussusception might, however, be envisaged, and it is necessary to establish whether or not intussusception takes place, by a direct measurement of the amount of cell wall formed under different conditions during the action of growth substance.

b. Methods

Sections cut from coleoptiles and placed in growth substance solutions of suitable concentration, elongate considerably, as shown in Table 14, which also shows that similar sections placed in water

(Insert Table 14)

alone elongate much less. The several conditions governing this elongation will be considered in detail in Part VI, but the fact that it does occur will be made use of at this point. These sections, since they have no external source of nutrient, must, if they form cell wall, accomplish it by a transformation of materials already

within the cell. Except for the material lost by respiration, their total dry weight must remain constant, and it is necessary to determine only that part of the total dry weight which may be considered as cell wall. For this purpose essentially the same method was used as in Part IV, i.e. the method developed by Hansteen-Cranner (1914) for the separation of cell contents from cell wall. The procedure was as follows; sections, in most cases 3.7 mm. long, were cut with a two bladed cutter from coleoptiles approximately 5 mm. below the apex. 10 such sections were placed in growth substance solution of the optimal concentration, i.e. 10 units per cc., and 10 sections into water alone. After the desired time of growth substance action the elongation of the sections was measured under a binocular microscope with a eyepiece micrometer. They were then placed in previously dried and weighed micro-gooch crucibles. The shape and dimensions of these crucibles are shown in Fig. VIII. In the bottom of each crucible had been placed a small mat of acid washed asbestos, the mat washed firmly into place, and the whole dried to constant weight at 95°. The sections, once in the crucible, were thoroughly ground against the sides of the vessel. They were then washed successively with 30 to 50 portions of cold water and an equal amount of boiling water. During the first few washings large amounts of colloidal material, presumably cytoplasm and cell sap, were removed. The later washings were clear, and the residue was then, according to Hansteen-Cranner and to the results of Part IV to be considered as cell wall. The cell walls were dried to constant weight at 95°. All weighings were performed upon a Kuhlmann micro-balance capable of estimating to 0.001 mg. The use of such a balance was necessary in view of the small amounts of material which it was desired to determine.

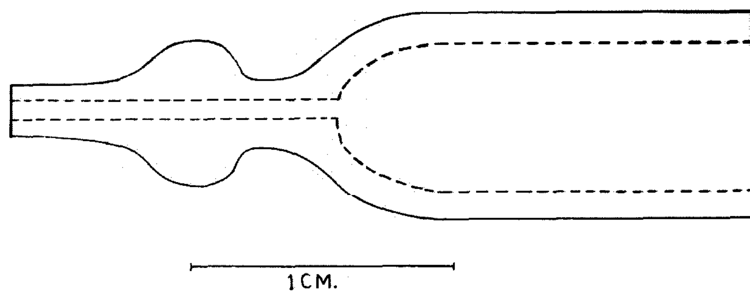

Figure 8. Micro-Gooch Crucible.

c. Experimental Results

A few series of duplicate determinations were first made in order to establish the accuracy of the method. In Table 15 it

(Insert Table 15)

may be seen that the agreement between duplicate determinations was in all cases quite satisfactory. In subsequent tables, then, only the means of the duplicate or triplicate estimations made in each case will be given.

If elongation is due to intussusception in the cell wall, one would expect the amount of cell wall per section to increase approximately in the same ratio as the length. Table 16 shows that

(Insert Table 16)

at 25°, this is in fact the case. In the mean the ratio of the wall weight of the two sets of sections having different elongations is almost exactly equal to the ratio of their lengths. This is a support of the assumption made in Part IV that at ordinary temperatures elongation is accompanied by wall formation. It will also be shown in Part VIII that at this temperature the total dry weight closely parallels coleoptile length over most of the coleoptile grand period. The fact that at 25° these processes fit well in with one another is reflected in the fact that this temperature is close to the optimum for the coleoptile.

Elongation is not of necessity, however, paralleled by formation of cell wall. Coleoptile sections were allowed to extend at 2°C. It was found that, as shown in Table 17, considerable elongation

(Insert Table 17)

took place at this temperature under the influence of growth substance if a preliminary hour at 25° was allowed. Under these conditions, altho 12 to 14 per cent of elongation took place, there was little or no difference in the wall weight of elongated and non elongated sections.

If growth substance solutions be made also 1 per cent in fructose, greater elongation takes place than in growth substance alone (see Part VI.). At the same time, as shown in Table 18 much

(Insert Table 18)

more cell wall is formed than corresponds to the increase in length. At 2°, however, fructose has no effect upon the formation of cell wall. The interest of this is two fold. In the first place it shows that the old supposition of Wortmann (see above) that decreases in elongation are due to formation of excessive cell wall cannot be in all cases correct. In the second place it shows again that cell wall formation bears no constant relation to elongation.

d. Conclusions

The results of the present section may be summarized as follows:

1) At 25° elongation may or may not be paralleled by cell wall formation, depending upon the nutrititive condition of the coleoptile.

2) At 2° cell wall formation ceases, altho elongation continues.

3) Active growth by intussusception of new material as a possible mode of growth substance action is therefore excluded.

Table 14.

Growth of Coleoptile Sections in Growth Substance Solution and in Pure Water.

(Each value is a mean of from 12 to 15 sections)

Solution	%Growth in 2 hours	% Growth in 4 hours	% Growth in 24 hours
Water	3	4	9
Water	3	5	7
Water	3	5	12
Growth Substance	15	24	55
Growth Substance	13	26	45
Growth Substance	14	29	48

Table 15.

Duplicate Determinations of Cell Walls

Crucible / Expt.	1.	2.	3.	4.
	Weight of Cell Walls per 10 Sections. Mgs.			
1.	0.75	0.73	0.75	0.69
2.	0.77	0.75	0.71.	0.70
3.	0.73	0.72	0.74	0.69
4.				0.71
5.				0.69

Table 16.

Increase in Cell Wall Weight During Elongation of Sections at 25°C.

(Growth Substance Action for 6 Hours.)

Expt.	Ln. in G.S. (Arbitrary units)	Ln. in Water (Arbitrary units)	%Growth in G.S.	%Growth in Water	Wt. in GS (Mgs.)	Wt. in Water
1.	4.19	3.80	13.2	2.6	0.714	0.609
2.	4.41	3.75	19.2	1.7	0.766	0.650
3.	4.50	4.01	21.6	8.3	0.865	0.766
4.	4.39	3.87	18.7	4.6	0.829	0.700
5.	4.56	3.88	23.3	4.9	0.847	0.758

Experiment	1.	2.	3.	4.	5.	Means
Length in G.S. / Length in Water	1.13	1.18	1.12	1.14	1.17	1.15
Weight in G.S. / Weight in Water	1.17	1.18	1.13	1.18	1.12	1.16

Table 17.

Increase in Cell Wall Weight During Elongation of Sections at 2°C. Growth Substance Action for 20 Hours.

Expt.	Length in G.S. (Arbitrary Units)	Length in Water (Arbitrary Units)	% Growth in G.S.	% Growth in Water	Weight in G.S. (Mgs)	Weight in Water (Mgs)	Ln. in G.S. / Ln. in Water	Wt. in G.S / Wt. in Water
1.	4.19	3.81	13.2	2.9	0.948	0.948	1.10	1.00
2.	4.16	3.85	12.4	4.2	0.944	0.911	1.08	1.03
3.	4.20	3.79	13.6	2.4	0.877	0.858	1.11	1.02
4.	4.19	3.79	13.2	2.4	0.838	0.843	1.11	0.99
5.	4.10	3.70	8.4	0.6	0.730	0.730	1.10	1.00
						Means	1.10	1.01

Table 18.

Increase in Cell Wall Weight During Elongation in Fructose and Growth Substance Solutions.

Expt.	Temp.	Length in G.S.	Length in Water	% Growth in G.S.	% Growth in Water	Weight in G.S. (Mgs)	Weight in Water (Mgs)	Ln. in G.S. / Ln. in Water	Wt. in G.S. / Wt. in Water
1.	25°	4.88	3.99	32	8	1.020	0.795	1.22	1.28
2.	25°	5.26	3.99	40	8	1.185	0.780	1.32	1.52
3.	25°	4.86	3.77	31	2	0.735	0.555	1.29	1.42
4.	25°	4.87	3.78	32	2	0.925	0.645	1.29	1.44
5.	2°	4.08	3.78	11	2	0.790	0.800	1.08	0.99
						Mean Ratios at 25°		1.28	1.42

PART VI.

CONDITIONS GOVERNING THE ELONGATION OF EXCISED COLEOPTILE SECTIONS

a. Introduction

The preceding sections have shown clearly that the effect of growth substance is not one upon intussusception of new material. Since, however, growth substance does influence the deformability of the coleoptile (Heyn, loc. cit.) the only alternative is that there is an increase in the plasticity of the cell wall. An effect upon plasticity might occur either directly upon the wall, as for example suggested by VanOverbeek (1933) or indirectly as a result of the action of growth substance upon functions of the protoplasm. The theory of VanOverbeck has been shown above to be untenable. It seems a priori highly unlikely that so few molecules of growth substance as are actually present in the plant should exert any direct effect upon the properties of the cell wall. One experiment which was done to investigate this possibility might be mentioned. If growth substance acts upon the wall directly, the only function of the protoplasm and cell contents would be to exert the necessary stretching force. Removal of turgor and its replacement by a comparable longitudinal tension should equally well bring about elongation under the influence of growth substance. The turgor of coleoptiles was removed by plasmolysis in KNO_3 and longitudinal tension replaced by 10 gram weights. According to Heyn (loc. cit.) such weights suffice to bring about considerable elongation of plasmolized coleoptiles. The coleoptiles carrying these weights were then suspended

in 3% KNO_3, elastic elongation permitted to cease and growth substance added to the solution. Not the least increase in elongation took place in the plasmolized coleoptiles, altho similarly suspended but turgid coleoptiles responded immediately. The most likely, altho not of necessity correct, or only explanation of this result is that the action of growth substance is upon the protoplasm and that the latter must be in contact with the cell wall in order to bring about the plasticity increase. In the present part it will be, however, rigorously proven that the action of growth substance does involve the intervention of functions of the protoplasm, especially of respiration. Before going to this it will be necessary to describe in detail some of the techniques used in the succeeding parts.

b. Methods

For the study of processes of a metabolic nature it is convenient to have the experimental object suspended in solution, the composition of which may be readily altered as desired. For this reason it was determined whether or not portions of coleoptiles would elongate in growth substance solutions. Table 19 has shown that they do and that the elongation in water alone is much less. The principal circumstances influencing this elongation will next be described.

The measurements of elongation were made in two ways; a) sections 3.7 mm. long were cut with a special two bladed cutter, and placed in the solutions. These sections could be measured conveniently with a 10x binocular and eyepiece micrometer to about 1.3 per cent. This is somewhat smaller than the variation between individual sections of a supposedly comparable series, as is shown

in Table 19. The individual variation is particularly large if

(Insert Table 19)

the average elongation is also large. A horizontal microscope equipped with a stand mounted upon a micrometer screw was used for a large portion of the measurements. Coleoptile sections were placed upon thin glass rods having a diameter just equal to the inside diameter of the coleoptile. These rods were in turn imbedded in paraffin inside of rectangular glass vessels through which the elongation of the sections, in this case originally 1 cm. long could be readily followed. No essential difference between the measurements carried out in these two ways was discovered. In all of the experiments about to be described the plants were decapitated two hours before using in order to obtain them relatively free of growth substance.

c. Elongation in Water Alone

The growth of sections of coleoptiles, prepared in the manner already described, and immersed in pure water was first investigated. Table 20 gives the results of a typical series of measure-

(Insert Table 20)

ments, and also a comparable series of measurements of sections with their bases in water, but their tips in air. It may be seen that the growth rate of the sections completely immersed in water falls steadily until after 6 hours it reaches a very low value. There is no sudden rise after two hours corresponding to a production of growth substance by the "physiological tip" as is the case with the sections whose tips were in air. The fact that any growth took place

in the sections immersed in water is to be attributed principally to growth substance in them when they were removed from the plants. Table 14 has shown that the elongation of sections in growth substance may be marked, being as large as 29% in four hours and 53% in 24 hours.

d. Stirring

It was conceivable that with these sections immersed in solutions, the rate of diffusion of growth substance to the cut surface which it enters, or the rate of diffusion of oxygen to the tissue, might prevent a maximum growth response. Therefore measurements of the growth rates of sections both with and without stirring of the solution by air were made. These results are given in Table 21

(Insert Table 21)

and show that stirring of the solution is not necessary.

e. Former position of Section in the Coleoptile

The effect of the original position in the coleoptile of a given section upon its response to growth substance was then investigated. That such an effect exists is shown by Table 22, which gives

(Insert Table 22)

the per cent growth per hour of sections from the tops and bottoms of a series of previously decapitated coleoptiles. Similar measurements made upon coleoptiles divided into more sections showed that the two 3.7 mm. sections nearest the apex of a 2-3 cm. decapitated plant have almost equal reactivity. The lower zones showed, as in Table 22, a lower ability to grow in the presence of growth substance.

It has been known from measurements upon the growth rates of entire coleoptiles (marked into zones with ink or paper marks) that the lower zones do grow more slowly than those nearer the top. This has been attributed, however, to a lack of growth substance in the lower zones which must receive it through a long portion of coleoptile actively using the growth substance. That this is not the only factor is shown here directly, since it is clear that the cells at the base of the coleoptile show a much smaller growth response to growth substance than those nearer the top. In the present work, unless otherwise stated, only the two sections from the top were used.

f. Growth Substance Concentration

The effect of concentration of the growth substance solution upon growth of the sections was determined, and is shown in Table 23.

(Insert Table 23)

From Table 23 it is evident that there is an optimum growth substance concentration in the region of 10 units per cc. Coleoptiles immersed in concentrations as great as 80 units per cc. show a shrinkage after 4 hours and at the end of 24 hours have frequently lost their turgidity due, apparently, to a toxic effect of the high concentration of growth substance.° A decrease in growth in very low growth substance concentrations was also found. A simple consideration will show that only in the case of the 0.01 unit solution can this be due to an insufficient quantity of growth substance, and that even in the 0.1 unit solution the decrease must be due directly to the low concentration. From the data of Part IV 0.00535 cc. of a 29 unit per cc. solution can give

° This toxity in high concentrations is not due to impurities in the crude growth substance preparations used here since crystalline growth substance "B" also exhibits toxicity in high concentrations.

under those conditions a maximum of 7.85 mm. of coleoptile elongation. 1 cc. of a 0.1 unit per cc. solution could then give a maximum of 5.05 mm. total coleoptile elongation. Since in general in the present case 12 sections were placed in 4 cc. of solution, these sections should be able to elongate a maximum total of 22 mm., or 59% per section, which is much larger than the 18% observed, a portion of which is, moreover, due to growth substance initially in the section. In the case of the 0.01 unit solution, however, each section should be able to elongate ~~only~~ 5.9% more than controls in pure water, and Table 23 shows that the increase is only 3% greater.

g. Fructose

The presence of a nutrient in the growth substance solution increases the elongation considerably. For this purpose fructose was found to give more consistent results than glucose. Table 24

(Insert Table 24)

gives an example of the effect of 1 per cent fructose upon the elongation. In general in the later experiments, however, fructose was not used since it proved quite favorable for the growth of microorganisms.

The pH of the external solution exerts a marked effect upon the growth rate of coleoptile sections. A detailed analysis of the effect of pH will be reserved for Part VII.

h. Inhibition of the Aerobic Metabolism

Upon the theory that the action of growth substance is a simple physical change of cell wall, for example by decreasing directly in some way the viscosity of the substance in which cellulose micelles

are imbedded (Heyn, 1931), one would hardly expect the action to be stopped by the presence of narcotics or cyanide. If, however, growth substance depends for its action upon processes of a metabolic nature, narcotics or cyanide should inhibit this action. It was easily demonstrated that both KCN and phenylurethane stop growth. Table 25 gives

(Insert Table 25)

~~gives~~ a summary of two experiments with different concentrations of KCN and of Phenylurethane and shows how marked is the stopping of growth. Further experiments will be given in Part VIII. It might be mentioned that potassium mono-iodoacetate as well as sodium fluoride also inhibit the elongation of sections.

Experiments were then carried out to determine if a connection between growth and cell oxidation exists. The action of growth substance in solutions under an atmosphere of pure nitrogen was first investigated. Commercial N_2 was passed over reduced copper in an electric furnace at 600°C. The gas was then cooled by passage through washbottles, and bubbled through the solution containing the coleoptile sections. A preliminary experiment showed that growth substance is not affected in its activity by prolonged passage of N_2 through it. The sections were freed of O_2 by treatment with N_2 for two hours before introduction of the growth substance solution. After this preliminary two hours, sufficient growth substance was introduced to make the solution 10 units per cc., and the bubbling was continued for four hours more. The sections were then measured, a portion of them placed in growth substance solution in air, and the remainder in pure H_2O, in air. Table 26 shows the results of 5 experiments.

(Insert Table 26)

The coleoptiles were not harmed by the prolonged lack of O_2 as is shown by the fact that they grew normally upon being supplied with both growth substance and oxygen. However, a mean growth of only 3% took place in N_2, although in air from Table 23 20% growth would take place. That even this 3% growth takes place is to be attributed probably to O_2 remaining in the sections. Therefore, normal growth fails to take place in N_2. Since the sections do not elongate, when placed in pure H_2O, to any extent more than if immersed in H_2O without the preliminary growth substance-N_2 treatment, either the action of growth substance does not take place or the growth substance is not taken up by the sections.

In order to show unequivocally that inhbition of aerobic metabolism inhibits actual elongation under the influence of growth substance, the following experiment was undertaken; two lots of sections were placed vertically upon glass pins; upon the apical ends of one set were placed agar blocks containing no growth substance. Upon the apical ends of the other set were placed blocks containing 1200 units per cc. The sections were then left at 25° in a saturated atmosphere for 2 hours. At the end of this time, a portion of the sections which had had no growth substance were placed in water, and the remainder placed in a 10 unit per cc. solution of growth substance. The plants which had had 1200 unit blocks were distributed among the following solutions:

a) KCN 3×10^{-3} N
b) KCN 1×10^{-3} N
c) Phenylurethane 0.05%
d) Water

Table 27 gives the results of one of five experiments which yielded closely similar results. There is no question but that growth substance passed into the plants which had 1200 unit blocks since these sections elongated more in water than did those which had plain agar, and were also placed in water. This elongation brought about by growth substance already in the plant is, however, clearly inhibited by both KCN and phenylurethane.

(Insert Table 27)

Table 27 shows that sections containing growth substance elongate when placed in water. Since the sections whose elongation in growth substance solution was inhibited by N_2 did not elongate to any great extent when subsequently placed in water they had not taken up growth substance. The uptake of growth substance as well as the actual action of growth substance is thus dependent upon aerobic metabolism.

i. Conclusions

In this section the technique of using excised sections of coleoptiles in the study of cell elongation has been described. The principal points are:

1) There is no "physiological regeneration" in sections immersed in water.
2) The original position of the section in the coleoptile greatly influences its subsequent response to growth substance. As would be expected, sections from the most rapidly growing zone of the coleoptile exhibit the greatest response.
3) There is an optimum growth substance concentration of 10 units/cc.

4) Fructose increases elongation in growth substance solution.

5) Elongation under the influence of growth substance is inhibited by KON, by phenylurethane, or by an atmosphere of pure nitrogen. It is demonstrated that both the uptake and the action of growth substance are connected with the aerobic metabolism.

Table 19

MEASUREMENTS OF SECTIONS WITH BINOCULAR MICROSCOPE

Section Number.	Original length mm.	Growth Substance 2 hours mm.	Growth Substance 8 hours mm.
1	3.70	4.00	4.25
2	3.65	4.00	4.35
3	3.70	4.10	4.35
4	3.70	4.35	4.75
5	3.75	4.20	4.50
6	3.70	4.25	4.65
7	3.70	4.10	4.35
8	3.65	4.00	4.25
9	3.75	4.15	4.50
10	3.70	4.10	4.35
11	3.70	4.00	4.25
12	3.70	4.00	4.10
Means	3.70 ± .01	4.15 ± .03	4.40 ± .05

Table 20

GROWTH OF COLEOPTILE SECTIONS IN PURE WATER

Expt.	Plants	% Growth per hr.					
		1 hr.	2 hr.	3 hr.	4 hr.	5 hr.	6 hr.
1 (Tops in water)	6	1.8	1.5	1.2	0.7	0.6	0.4
2 (Tops in air)	6	2.5	2.5	4.0	4.3	4.7	3.3

Table 21

GROWTH RATES OF COLEOPTILE SECTIONS IN
GROWTH SUBSTANCE SOLUTION WITH AND WITHOUT STIRRING BY AIR

Expt.	Sections	% growth per 2 hr. after					Total %
		2 hr.	4 hr.	6 hr.	8 hr.	10 hr.	24 hr.
1 (no air)	7	8	6	3	1	0.2	16
2 (no air)	6	9	4	3	2	0.3	19
3 (no air)	7	7	3	2	---	---	16
4 (air)	5	7	5	2	---	---	22
5 (air)	7	6	4	1	1	2	14
6 (air)	7	8	5	3	---	---	21
Mean of 1, 2, and 3		8.0	4.3	2.7	---	---	17.0
Mean of 4, 5, and 6		7.0	4.7	2.0	---	---	19.0

Growth substance = 10 u/cc

Table 22

GROWTH RATES OF TOP AND BOTTOM SECTIONS OF COLEOPTILES IN
GROWTH SUBSTANCE SOLUTION

	% Growth per hour						
	1 hr.	2 hr.	3 hr.	4 hr.	5 hr.	6 hr.	7 hr.
Top sections	4.5	4.2	4.3	3.7	2.7	2.4	2.4
Bottom sections	1.0	1.0	1.1	1.4	2.1	0.9	0.9

Growth substance = 10 u/cc

TABLE 23

EFFECT OF GROWTH SUBSTANCE CONCENTRATION UPON GROWTH OF COLEOPTILE SECTIONS

(Each value mean of 50-150 sections).

Growth Substance concentration Standard Units.	% growth in 2 hours	4 hours	24 hours
80	3.3	2.1	0.4
40	4.3	8.0	7.2
20	7.4	10.8	15.4
10	11.7	19.9	31.0
1	8.4	15.7	27.0
0.1	6.5	12.1	17.5
0.01	4.5	7.0	15.5
0.00	3.3	5.6	11.9

TABLE 24

EFFECT OF FRUCTOSE ON GROWTH OF SECTIONS

Expt.	Solution	Growth in % 4 hr.	8 hr.	24 hr.
1	1% fruc., 10 u/cc	---	23	27
	10 u/cc	11	13	16
	1% fruc.	---	9	14
	H_2O	4	7	9
2	1% fruc., 10 u/cc	12	23	33
	10 u/cc	10	14	15
	1% fruc.	2	3	6
	H_2O	2	3	6
3	1% fruc., 10 u/cc	13	21	35
	10 u/cc	14	18	20
	1% fruc.	5	7	10
	H_2O	6	7	8

TABLE 25

INHIBITION OF GROWTH OF COLEOPTILE SECTIONS BY
KCN AND PHENYLURETHANE

Solution	% Growth	Solution	% Growth
g. s. alone	23	g. s. alone	20
g.s. + $2x10^{-4}$ N KCN	5	g.s. + 0.001% phenylurethane	23
g.s. + $1x10^{-3}$ N KCN	2	g.s. + 0.01% "	14
g.s. + $2x10^{-3}$ N KCN	- 4	g.s. + 0.1% "	2
g.s. + $2x10^{-2}$ N KCN	- 3	H_2O. + 0.1% "	3
H_2O + $2x10^{-2}$ N KCN	- 4		

TABLE 26

INHIBITION OF GROWTH OF COLEOPTILE SECTIONS BY NITROGEN

Expt.	No. of Sections	% Growth after 4 hr. in N_2 + g.s.	% Growth after 20 hr. in air+g.s.	% Growth after 20 hr. in air+H_2O
1°	20	4.5	17	—
2	26	2.9	21	—
3	25	1.7	—	3.8
4	33	1.4	28	8.0
5	46	4.5	13	4.5

°In this experiment the sections were not first freed of O_2.

TABLE 27

INHIBITION OF ELONGATION (AFTER GROWTH SUBSTANCE UPTAKE) By KCN AND PHENYLURETHANE

Agar Block (2 hr.)	Subsequent solution	Sections	% elongation during block action	Subsequent % elongation during 4 hr.	8 hr.	20 hr.
water agar	water	15	3.0	2.1	3.4	6.1
water agar	10 u/cc	15		18.1	27.6	33.2
g.s. agar	$3x10^{-3}$N KCN	15		1.8	2.1	3.9
g.s. agar	$1x10^{-3}$N KCN	15		2.4	3.7	5.0
g.s. agar	0.05% Phenyl-urethane	15		4.1	4.9	5.5
g.s. agar	water	15	5.0	11.2	12.8	25.5

PART VII.

THE RELATION OF HYDROGEN IONS TO ELONGATION

a. Acid Growth and Acid Curvatures

The study of cell metabolism in relation to growth substance was commenced with an investigation of the relation of hydrogen ions to growth from the following considerations. It has long been known (for example Borowikow, 1913) that acids may considerably increase the growth rate of plants immersed in them. This fact has been elaborated upon by Strugger (1932, 1933, 1934) and extended into a general theory of cell elongation. According to Strugger, suitable increases of acidity, either by cellular metabolism or by experimental interference, increase the imbibition pressure of the protoplasm and this in turn brings about an increase in growth rate. As pointed out in the general introduction there is no good reason to believe that an increase in the imbibition pressure of the protoplasm would, as such, have any marked effect upon growth rate. It remains, however, possible that changes either in cell wall hydration or in some other property of the protoplasm determined by its pH may influence the growth rate. It seemed desireable therefore to investigate the whole problem of the relation of acidity to elongation with a view not only to the theory of Strugger but also in order to discover any possible connections with growth substance.

Strugger has described the effect of acids, in certain concentrations, in increasing the growth rate of _Helianthus_ roots and hypocotyls. The growth rates of _Helianthus_ roots are, according to Strugger, minimal when they are placed in buffers of pH about 5.1.

Upon each side of this minimum the growth rates rise to a maximum and fall again to low values at pH 3 and pH 8. The curve of growth rate against pH shows then two maxima. By removal of strips of epidermis of one side of hypocotyls, and by then placing these hypocotyls in buffers of suitable pH, Strugger was able to cause large curvatures away from the point of entry of the buffer. Buffers which caused fast growth caused large curvatures, and this was interpreted as signifying that the parenchyma of the side near the point of entry of the acid grows faster than the parenchyma of the opposite side. These curvatures will be called for convenience "acid curvatures" in the following discussion. Strugger advanced the hypothesis that cell elongation is intimately connected with the pH of the cell protoplasm relative to its isoelectric point. According to this hypothesis, roots and hypocotyls show a minimum growth rate at pH 5.5, because this is the isoelectric point of their protoplasm. With change of acidity on either side of this point an increase of growth rate results.

Dolk and Thimann (1932) found that if growth substance is supplied to the Avena coleoptile in a solution buffered at an alkaline pH its effect in causing cell elongation is abnormally small. It is a matter of general experience both in this laboratory and in others (Boysen-Jensen, 1932; Kögl and HaagenSmit, 1931), that solutions of growth substance must be acid in order to give the maximum effect upon cell elongation. This fact has suggested that there might be some relation between growth substance and the "acid growth" of Strugger. Such a relation, if found to exist, could perhaps lead to some elucidation of the mechanism of the action of the growth substance itself. The present part deals therefore with the "acid growth" and

"acid curvature" under the following heads:

a) The nature of the phenomenon.
b) The acidity of the coleoptile during growth.
c) The dependence of acid growth on growth substance.

b. The Nature of the Phenomena

If an entire section of a growing root, hypocotyl, coleoptile, or other organ is placed in a certain solution, the effect of this external solution upon the growth rate of the organ will depend greatly upon the speed with which the solution penetrates from the cut ends to the interior cells. The epidermis with its cutinized wall is relatively impermeable. Hence a small effect of a dissolved substance upon growth rate does not demonstrate unabiguously that the substance has a small effect upon the growth rate of a single cell. It is necessary to distinguish between transport and action of the substance concerned. It is possible to simplify the problem of transport to a certain extent by the removal of the epidermis from one or both sides of the organ. The dissolved substance, acid, for example, may then penetrate directly the entire length of parenchyma. If acid accelerates the growth rate of the parenchyma, and if the epidermis is removed from only one side, a curvature away from the side without epidermis will result, because the cells nearest the wound will increase their growth rates before those on the side away from the wound. Such a curvature will, however, tend to disappear after a time when all of the cells have been reached by the acid and elongate at the same rate. If, however, the parenchyma increases its growth rate without a corresponding increase in the growth rate of the epidermis, a permanent curvature will result since

epidermis hinders growth on one side and not on the other. This, as will be shown, is what occurs in the case of an _Avena_ coleoptile which has had its epidermis removed from one side and which is placed in a sufficiently acid buffer solution.

Methods. For carrying out of acid curvatures, coleoptiles (depapitated 2 hr. before) were cut off from their mesocotyls and placed in rows upon short glass rods having diameters equal to the inside diameters of the hollow coleoptiles. These rods penetrated, of course, only a mm. or two into the lumen of the plants and in no way interfered with the curvatures which were obtained. The rods were in turn mounted upon glass strips (10 rods per strip) and the entire arrangement could then be placed in a petri dish containing the solution under investigation. The removal of strips of epidermis was easily executed by merely stripping the latter off with a pair of small forceps. The strips which were removed were about one half mm. broad and were always taken from one of the narrow sides of the slightly elliptical coleoptile. At the expiration of the experimental period shadow photographs of the resultant curvatures were taken through the petri dish. It should be mentioned that altho the coleoptiles were placed horizontally and altho they are normally very sensitive to geotropic stimuli, they did not under these conditions exhibit any geotropic response whatsoever, and remained quite horizontal. The strips of epidermis which were removed were not of constant breadth and this caused a variation of the curvatures given by a series of otherwise rather comparable plants. Table 28 confirms

(Insert Table 28)

Strugger's observation of acid curvatures and shows the extent of

this variation in two series of plants, one with large angles and one with small angles. Because of the rather large errors, it is of course necessary to base conclusions upon several experiments. In the following discussion curvatures away from the side without epidermis will be designated as –. McIlvaine's phosphate, citric acid buffers were used since they make possible the use of the same materials over a wide range of pH. The buffers were used, at different times, between the molalities of 0.0025 and 0.1 and were, hence, considerably below the osmotic value of the cells, which are in equilibrium with about 0.25 molal KNO_3.

Cell wall plasticity and elasticity were measured in the manner described by Heyn (1931). The coleoptiles were fastened horizontally by their bases on short glass rods, which were in turn mounted in vertical rows on supports. At uniform distances from the end of the supports, weights of 250 mg. were placed and fastened with a small amount of lanolin. The coleoptiles were then placed in a saturated atmosphere under a bell jar to prevent drying. Preliminary experiments showed that if the riders were allowed to remain one half hour, large differences in the curvatures of the plants infiltrated with different buffers developed. Shadow photographs were taken, the weights removed, and the curvatures photographed again in two minutes. The curvature remaining after removal of the weights, is the plastic portion, i.e. that due to irreversible stretching of the cell walls. The difference between the curvatures when the weights are still upon the plants and the plastic portion is the elastic or reversible curvature.

Experimental. The acid curvatures appear in as short a time as 20 minutes and after 2 hours they change relatively little (Table 29). For comparative purposes, then, all curvatures were

(Insert Table 29)

measured after two hours. These curvatures were very large if the plants were placed in a buffer of pH 4.1 or less. External acidities of greater than pH 4.1 proved to be toxic after more than two hours. Plants placed in buffer solutions of pH 7 showed little if any curvature. The cause of these curvatures will now be considered.

It has already been pointed out that in order for a permanent curvature to result it is necessary that there be a difference in elongation of epidermis and parenchyma. It was even conceivable that the curvatures might be due to an active contraction of the epidermis. The behavior of isolated strips of epidermis in buffers of different pH was therefore determined. The growth rate measurements were made with a horizontal microscope and rectangular glass vessel in the manner previously described. Epidermis was obtained by the removal of strips about one half mm. broad from ordinary plants. These strips were fastened to small hooks with aid of drops of warm wax, and were then suspended in the observation vessels. One-half gram weights were placed on the bottom ends of the strips to keep then under tension. Table 30 shows that by comparison with the

(Insert Table 30)

growth of plants having much of the epidermis removed (see below) the growth of the epidermal strips is very slight; neither does this growth depend particularly upon the pH of the medium. However, the epidermis undergoes no shortening, and therefore this hypothesis

concerning the acid curvatures is at once ruled out. It was then necessary to look for a difference of growth of the parenchyma in acid and in neutral solutions.

In order to have "parenchyma" as comparable as possible with the parenchyma of the plants of the curvature experiments the following device was used. The epidermis was removed as equally as possible from two sides of the plants to be used. Since any small asymmetry in the remaining epidermis was found to cause curvatures which prevented accurate measurement of the longitudinal growth, the plants were placed upon thin glass rods which traversed the entire lumen of the hollow coleoptile. This arrangement effectually prevented curvatures without having any appreciable effect upon the growth rate. Table 31 compares the growth rate at pH 4.1 and at pH

(Insert Table 31)

7.2, and the results presented are characteristic of a large number of measurements. In buffer of pH 7.2 the growth is small and of the same order as that shown by Table 30 for the epidermis. Under these circumstances, then, no curvatures would be expected. In buffer of pH 4.1, the growth during the first two hours is 14 times that in pH 7.2. It may also be observed that the principal portion of the growth takes place during the first 2 hours, just as was shown to be the case for the curvatures (Table 29).

The acid curvatures are thus found to be due to difference in growth of epidermis and parenchyma. In solutions which are neutral or nearly so curvatures do not occur, because the growth of the parenchyma is slowed down to about that of the epidermis.

<u>Effect of acid on the properties of the coleoptile.</u> There are various ways in which acid might bring about its effect on the growth rate. Among the possibilities are:

a) a swelling of the walls of the parenchymatous cells;

b) an increase in the water permeability of the parenchymatous cells, by which an increased rate of water uptake would be made possible;

c) an increase in the plasticity of the cell walls.

If a "swelling" of the walls of the parenchyma is responsible for the acid growth reaction, curvatures should result as well in plasmolized as in unplasmolized coleoptiles. The epidermis of young coleoptiles was removed from one side. These plants were placed in 10% KNO_3 solutions which at the same time were buffered at pH 4.1 and 7.2. Table 32 shows that there is no large curvature in either set

(Insert Table 32)

of plants. It appears therefore that the acid growth reaction depends upon the maintenance of turgor pressure.

The rate of deplasmolysis in hypotonic solutions may be taken as some measure of water permeability. (See the recent critical review by DeHaan, 1933). In the present case coleoptile sections were allowed to plasmolize in 10% KNO_3 and then to deplasmolize in the usual buffers of pH 4.1 and 7.2. The deplasmolysis took place on a welled slide, and was followed under the microscope. In both cases the rate of deplasmolysis was very rapid, most of the cells being completely filled out in less than a minute. There was no observable difference between the behavior in pH 4.1 and that in pH 7.2. It is difficult to see how any small difference which may

exist between cells so extremely permeable to water as these, can account for differences in growth rate.

The effect of acid on cell-wall plasticity was next investigated. In order to avoid ambiguous effects of injury as well as the longitudinal transport problem, the coleoptiles were in this case infiltrated with the solutions by means of a vacuum pump. Table 33

(Insert Table 33)

shows that infiltration does affect the initial growth rate in the same manner as does removal of the epidermis. After a time of action of the buffer solutions of 1 to 4 hours, cell wall plasticity and elasticity were determined in the manner already described. Table 34 shows the results of five experiments which demonstrate clearly

(Insert Table 34)

that under the present conditions, the difference in the action of pH 4.1 and pH 7.2 is accompanied by a considerable difference in the plasticity of the cell wall, i.e. they are more plastic in the acid solution. In every case the difference in the elastic portions of the curvature is small. The effect of acid upon plasticity is to be stressed since it closely resembles the effect of growth substance upon plasticity which has been described by Heyn.

It has often been attempted to determine whether curvatures, either tropistic or as in Strugger's case, due to acid solutions, are due to "active growth" or to elastic bending, by measuring the portion of the curvature which is removed upon plasmolysis. Tröndle (1917) and Overbeck (1926) found that the first stages of tropistic curvatures disappear upon plasmolysis whereas the later, larger

curvatures are irreversible. Strugger concluded that since his acid curvatures were only slightly reversible by plasmolysis that they concerned "dabei keinesfalls eine blosse Überdehnungserscheinung, sondern es liegt wirkliches Membranwachstum durch Intussusception vor". The irreversibility of a curvature upon plasmolysis does not, however demonstrate that the curvature was due to growth by intussusception. In the first place, irreversible curvatures may also be obtained by plastic elongation with or without accompanying intussusception. In the second place, in the Avena coleoptile at least, the curvatures which remain after plasmolysis are complicated by the tension which exists between epidermis and parenchyma before plasmolysis. Measurements showed that incoleoptiles of the standard length (2 to 3 cm.) the epidermis contracts 10 to 15 per cent if it is removed from the plant (see also Heyn, 1933). Normally therefore it is under considerable tension. If perfectly straight coleoptiles having the epidermis removed from one side are plasmolized they sometimes show negative curvatures of as much as 55 degrees due to the greater contraction of the remaining epidermis than of the parenchyma. It is not safe to conclude, without an examination of this factor of tissue tension, that a curvature is plastic or that it is due to intussusception by the fact that it is not removed upon plasmolysis.

c. Acidity of the Coleoptile during Growth

Method. Colorimetric determinations of the cell sap showed that under normal conditions the pH is in the neighborhood of 6. However, colorimetric determinations did not permit of sufficiently accurate measurements of small differences which might exist between portions

of the coleoptile having different growth rates. Therefore electrometric methods were resorted to. The quinhydrone electrode was used. The advantages of this electrode over the hydrogen electrode for the present purpose have been pointed out by Gundel (1933). Briefly they are:

a) since H_2 is strongly reducing in the presence of platinum black, relatively large changes of the oxidation-reduction potential of the sap may be caused, and this is partially avoided by the use of the quinhydrone electrode;

b) the passage of H_2 through the sap drives out CO_2 and may change the pH if the carbonate buffer system is of importance.

Considerable difficulty was experienced at first in obtaining reproducible platinum electrodes. However, the following method of cleaning the electrodes was found to be satisfactory. They were first dipped for a few minutes in hot aqua regia and then boiled for several minutes in a solution of sodium bi-sulfite. With this method of cleaning, the three electrodes which were used for each determination agreed in general to within 1 milli-volt. A calomel-saturated KCl reference half-cell and a saturated KCL bridge were used. Frequent checks of the potential of the quinhydrone cell were made by means of standard HCl solutions and always gave within 1 millivolt of the expected value. The potential of the cell sap was found to change with time. The sap appeared to become more acid. This drift was reduced, altho not completely eliminated, by heating the coleoptiles to 100° C. for 2 minutes before crushing them. For comparative purposes, therefore, the readings after 10 minutes were arbitrarily taken. For comparison, a few measurements were made with the micro glass electrode (McInnes, 1929).

The determinations were carried out in the following manner. 25 to 30 3.1 mm. coleoptile sections were heated over a boiling water bath for two minutes. They were then ground in a porcelain dish, diluted with an equal volume of water (which was found to cause no significant change of the potential), a small amount of Eastman quinhydrone added, the vessel put in place in a 25° C. thermostat, connected with the KCl bridge, and the potential with each of 3 platinum electrodes determined with a Leeds and Northrup type K potentiometer.

Experimental. For a comparison of rapidly growing and slowly growing zones of the plant, the coleoptiles were decapitated and the following portions removed:

a) the 3.1 mm. zone beginning about 5 mm. from the tip (zone of maximum growth rate; Went, 1928);

b) the 3.1 mm. zone next to the mesocotyl (zone of minimum growth rate, except, of course, for the extreme apex).

Table 35 gives the results of a number of measurements. In each

(Insert Table 35)

experiment a considerable amount of variation is found among samples which would be expected to be the same. This is probably due to reaction of the quinhydrone with the metabolites of the sap, since the determinations made with the glass electrode (where reactions of the metabolites with the measuring system are excluded) were rather more constant. Table 35 shows that the "tops" are slightly altho not significantly more basic than the "bottoms". This result is in accord with general experience, altho the difference is smaller than has been sometimes observed. Sachs, for example, found in 1862 that the most apical portion of a growing root is basic, the zone of maximum

growth rate "almost neutral" and the more basal portions "decidedly acid". Gundel more recently found that in the roots of <u>Vicia faba</u>, hypocotyls of <u>Helianthus</u>, and epicotyls of <u>Phaseolus</u>, there is an increase of acidity in the older portions.

It is not safe to conclude, from the above only, that there is no change of pH during the action of growth substance. As shown by Thimann (1934) the upper zone contains more growth substance than does the lower and this is as would be expected, since it is nearer the source, i.e. it is nearer to the tip. However, as has already been pointed out even with the same growth substance supply the lower zone grows more slowly. A comparison of the same zone with different growth substance supplies was therefore made. Solutions a) of 1% fructose and 10 units growth substance per cc. and b) water alone were used. Table 36 shows that again the pH of the rapidly growing

(Insert Table 36)

sections is slightly, altho hardly significantly more basic than that of the slowly growing sections. This conclusion was again corroborated with measurements made with the glass electrode.

It is impossible to correlate an increased rate of cell elongation due to growth substance action with an increase of cell acidity, at least so far as measurements made upon crushed cells go. The pH which has been determined is mainly dependent upon that of the cell sap, but this sap is mixed with the protoplasm as well as with the cell walls. How far the pH of the protoplasm and cell walls affects that of the sap cannot be said, but it seems likely that great differences between the protoplasmic acidity of fast growing and slow growing sections would be reflected by at least a small

change in that of the poorly buffered sap. Whatever difference is found, however, lies in the opposite direction from that which would be expected if pH changes play any part in the control of normal growth.

Increase of cell acidity is not, then, a prerequisite to or an accompaniment of normal growth. The significance of the acid growth reaction is therefore to be sought elsewhere. This will be considered in the following section.

d. The Dependence of Acid Growth on Growth Substance

If one considers the relation of acid growth to the growth normally brought about in the *Avena* coleoptile by growth substance, the various possibilities may be included under the following heads:

a) acid action is entirely unrelated to that of growth substance;

b) acid acts by bringing about the same cellular changes as does growth substance, but short circuits the steps for which growth substance is necessary;

c) acid acts through an influence upon growth substance which then causes growth in the normal manner, whatever this may be.

The first possibility will be neglected for the time being. Strugger seems to consider the second possibility as likely. He found that under all conditions the objects with which he worked showed a much increased protoplasmic viscosity with acidity, and considered that the increase in viscosity and increase in imbibition pressure leads to an increase in growth rate. In the course of the present study the protoplasmic viscosity of *Avena* coleoptiles was studied by *Weber's* plasmolysis form and time method (the method used by Strugger). The viscosity was found to remain, within the

rather considerable uncertainty of the method, unchanged during cell elongation, and to be independent of the action of growth substance. In the case of Avena, then, large changes of protoplasmic viscosity are not of necessity associated with growth (altho they sometimes occur; see Lange, 1933) and the general significance of such changes may consequently be questioned.

It was shown in a previous section that at least one of the ultimate effects of acid upon the coleoptile is identical with that of growth substance, namely an increase of cell wall plasticity. It has also been shown above, however, that the action of growth substance probably does not involve a production of acid in the cell. Therefore the supplying of acid to the cell does not result in a simple shortcircuiting of the processes for which growth substance is necessary. More complicated actions of acid in shortcirciting these processes might be conceived. It will now be shown, however, that in the case of the Avena coleoptile at least, placing the plant in an acid medium results in the conversion of growth substance already in the cell from a relatively inactive ionized form to an unionized form which is active in causing cell elongation, and that "acid growth" is completely dependent upon growth substance.

(a)

If agar blocks are placed upon one side of decapitated coleoptiles, curvatures result, which are within certain limits proportional to the amount of growth substance in the blocks (Went, 1928). However, as previously pointed out, it was found by Dolk and Thimann (1932), that if the agar block containing growth substance is buffered at a neutral or alkaline pH the curvatures are much smaller than would

be expected. For example, from their unpublished data, a growth substance solution which when acidified and made into agar blocks gave 12.6 degrees of curvature; when made into agar blocks with buffer of pH 7.0 gave only 3.3 degrees. It follows that growth substance solutions must be made acid in order to obtain large effects upon growth. The reason for this is, as already mentioned, that growth substance is a weak acid and the undissociated form has apparently a greater growth potency than does the salt. Table 37 compares the growth rates of plants (with their epidermises removed) in several solutions. It may be seen that

(Insert Table 37)

a) growth substance in acidified but unbuffered solution greatly increases the growth rate over that in distilled water;

b) growth substance in acid buffer (pH 4.1) causes an even further increase.

c) in buffer of pH 7.2 the growth rate is very small and is not greatly increased by the addition of growth substance.

In brief, growth substance is of considerable use to the plant only when it is given in the undissociated form. This provides a basis for the effect of acid upon growth if it can be shown that there is dissociated growth substance in the plant which can be converted into the active form.

(b)

The earlier attempts to extract growth substance from the lower portions of coleoptiles have always given negative results. This, as is now apparent, is because the amount present is rather small, and the usual technique, that of placing the plants upon agar

blocks and allowing the growth substance to diffuse out (Went, 1928) does not permit of the accumulation of enough plants on one block to give a readily detectable amount. In order to obtain amounts which may be determined by the curvature assay in the usual manner, it is necessary to grind up a large number of plants, extract the pulp, and concentrate the solution. The work of Thimann (1934) has shown that if chloroform is used as the solvent, and the solution is acidified during the extraction, growth substance may be readily obtained from coleoptiles. Table 38 shows that about 5 plant units per plant are obtained in this way, and this agrees with

(Insert Table 38)

the amounts found by Thimann. The point of interest in the present connection is that there is extractable growth substance present in the plant. It has already been shown that normally about 94 per cent of this growth substance will be in the inactive or slightly active salt form (because of the pH of the plant). It will later be shown that when a coleoptile is placed in an acid solution its internal pH decreases, although not to that of the external solution. Therefore more of its own growth substance is available to the plant when it is placed in such an acid buffer.

(c)

The minimum of growth rate reached by plants after decapitation has been interpreted as due to the using up of the residual growth substance after removal of the source (the tip) and a subsequent "physiological regeneration" of the tip. According to this view, acid curvatures made with freshly decapitated plants should be

larger than those made with plants decapitated 2 hours previously. Table 39 shows that this is actually the case.

(Insert Table 39)

(d)

The per cent inhibition of "acid growth" and of "growth substance growth" by HCN is of the same order. Table 40 gives the

(Insert Table 40)

mean results from several experiments in which the growth rates of plants (with the epidermis removed from the two sides) in various solutions is compared. It will be seen that the change of "acid growth" with time resembles that of "growth substance growth". Growth substance gives in the first hour, however, considerably more growth, than does acid. In comparing the growth rates in HCN of various concentrations, only the first hour may be safely used, since damage and loss of turgor may result after this time. From Table 41 it may

(Insert Table 41)

be seen that the two kinds of growth are stopped by HCN concentrations of the same order altho the acid growth is somewhat less sensitive than is the growth substance growth. Other experiments have also indicated that the uptake of growth substance is more sensitive to HCN than is the action, and the acid growth, interpreted as due to growth substance is concerned only with the action of growth substance already present in the individual cells. Hence we find a somewhat smaller sensitivity to HCN in the case of the acid growth. The acid curvatures are also partially altho not completely inhibited by HCN.

(e)

The change of acid curvatures with pH may next be considered. Table 42 gives the results of 5 typical experiments over the range

(Insert Table 42)

from pH 2.5 to pH 8. The trend of the curvatures is the same in all of the experiments but the abolute values of the angles vary considerably. This variation, the cause of which is undetermined, occurred in all of the large number of experiments which were carried out. The mean values from all of the experiments do not differ significantly from those of the five experiments of Table 42, and for convenience only the latter will be considered here. The change of curvature with pH is illustrated graphically by Fig. IX, curve B. In contrast with the above mentioned double maximum curve obtained by Strugger for Helianthus roots, this curve is of an S type, and resembles rather strikingly the titration curve of an acid. Fig. IX (curve A) gives also the titration curve of an acid of pK 4.8) that of growth substance; Dolk and Thimann, 1932). In order to compare the two curves it is convenient to reduce them to the same ordinate at some particular pH, and to observe how they change on either side of this point. Curve C is constructed so that at pH 6.1 (that of the cell sap) the percentage curvature is 4.2 or equal to the per cent of undissociated acid of pK 4.8 remaining in a solution of pH 6.1. Suppose that as already indicated the acid curvatures are due to transformation, at some point inside of the plant, of growth substance from an inactive salt form to active acid form, and that the active growth substance thus produced causes the growth which results in curvature. We would expect that the pH in the plant would not be

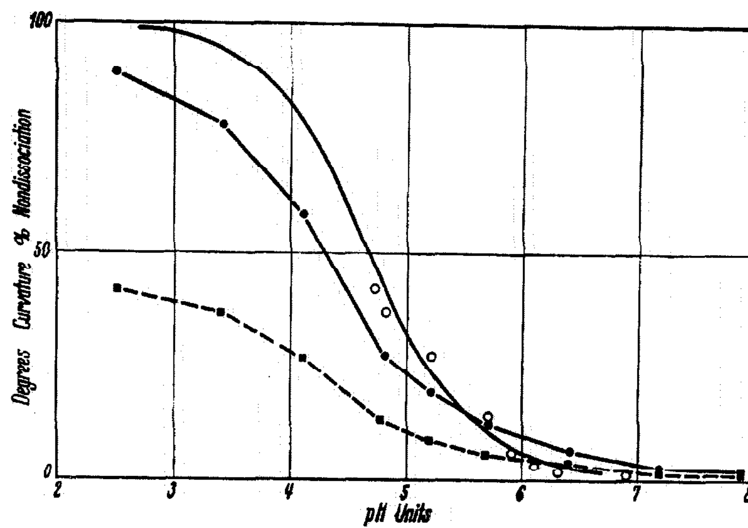

Figure 9.Relation of Acid Curvatures to pH.

—— Titration Curve of Acid Having pK 4.8 (Curve A)

•—— Curve B.Change of Actual Curvature with pH.

■ - - Curve C,% Change of Curvature (See Text.)

exactly that of the outside buffer solution, since the buffer has first to penetrate the plant, and then to overcome whatever buffering capacity the plant may possess. Therefore, the per cent curvature should not change as rapidly with pH of the external buffer as does the per cent non-dissociation of an acid dissolved in the buffer itself. This is just what is found in Figure IX, at least, so far as the acid side of pH 6.1 is concerned. Upon the basic side the curvatures are so small that it is impossible to draw any conclusion. It was possible to show directly that the acidity inside of the plant does not change exactly with that of the buffer. This was done by measuring colorimetrically the pH of the crushed coleoptiles (previously washed free of buffer left upon the surface) after the execution of acid curvatures. Table 43 gives a characteristic set of these values together

(Insert Table 43)

with those expected from Figure IX. If these points are plotted on Figure IX, they are seen to lie reasonably close to the titration curve. If the actual acidity of the plant is considered, then the per cent curvature increases as it should if it is the formation of unionized growth substance which causes the curvature.

e. Discussion and Conclusions

It is difficult to reconcile the present findings with those of Strugger for *Helianthus*. In the case of *Avena* the change of acid growth rate with pH is determined by the change of the dissociation of the weak acid, growth substance. In *Helianthus* according to Strugger the change of acid growth with pH gives a double maximum

curve between pH 3 and pH 8. Borowikow (1913) who also worked with <u>Helianthus</u> obtained results which may be more easily understood in the light of the present work. He found large stimulations of growth in acid solutions. These stimulations were decreased by the addition of neutral salts to the solution. The stimulation given by bases on the other hand was either small or absent and in general, rather sporadic in its appearance. He explained this, since he felt that it would be suitable to find two maxima, as due to the fact that a basic solution with a growing plant in it is always a mixture of salt and base because of the CO_2 evolved by the plant. His results might equally well be explained as due to the absence of any particular stimulation by bases. In the course of the present work a few measurements of the acid curvatures of both <u>Helianthus</u> hypocotyls and <u>Pisum</u> epicotyls have been made. Table 44 shows that under the

(Insert Table 44)

present conditions the same general change of curvature with pH takes place as was found for <u>Avena</u>, and the different results of Strugger are probably due to a fundamental difference of technique. The behavior of <u>Helianthus</u> is according to Strugger different in other respects from that of <u>Avena</u>. He believes to have found that hypocotyls grow faster under anaerobic conditions than in the presence of air, and has interpreted this as due to the production of acids during anaerobic metabolism. This is, as already shown, the reverse of the situation in <u>Avena</u>. It has also been impossible to find the changes in protoplasmic viscosity which Strugger has associated with the pH and isoelectric point of the protoplasm.

The work of the present section was undertaken with the hope that it might lead to an elucidation of the manner in which growth substance acts in bringing about growth. This elucidation would have been possible if the effect of acid had been, as mentioned above, to bring about the same cellular changes as does growth substance, but with the shortcutting of the steps for which growth substance is needed. The facts presented show, however, that the effect of acid on growth is, in the case of Avena at any rate, only by way of the effect of acid on growth substance already present in the plant. It is also shown that in the normal plant, as well as in the action of growth substance upon sections cut from the plant, there is no increase of acidity as the result of or during the action of growth substance. It would appear then that there are at least two ways connected with growth substance in which the growth rate of an Avena coleoptile can be increased. The first is the way in which it is done in the normal plant, by an increase of the total amount of growth substance present. Only a small portion of this will be in the active non-dissociated form at the pH of the plant, but by increasing the total amount present the amount present as non-dissociated acid will also be increased. The second way in which the growth rate may be increased is by conversion of some of the inactive growth substance salt already present in the plant into the acid. This is what occurs in the "acid growth" reaction, when the cells of the plant are actually made more acid, and the ionization of growth substance reduced. It remains possible that there are some other effects of acid upon the coleoptile but it is clear that so far as the growth rate is concerned these other effects, of which a small change in protoplasmic viscosity may be one

are of minor significance under the present conditions. The different results of Strugger, due perhaps to differences in material or other experimental conditions, are probably due to the elevation of other factors to importance.

The results of the present section may be summarized as follows:

1) The growth of the _Avena_ coleoptile in buffer solutions of different pH has been investigated. The formation of curvatures in acid solutions by plants having the epidermis removed from one side has been confirmed.

2) Acid solutions increase the growth rate by increasing the cell wall plasticity. Increase of cell wall plasticity is known to result from the action of growth substance.

3) The increase of growth rate in acid buffers is inhibited by HCN concentrations of the same order as those which inhibit the increase of growth rate due to growth substance.

4) There is no increase in cell acidity under the influence of growth substance.

5) There is growth substance present in the plant even after the hormone producing tip is removed.

6) The increase of growth rate with decrease of _internal_ pH follows closely the increase of non-dissociated acid with decrease of pH in a solution of the weak acid, growth substance.

7) From the foregoing it is concluded that the increase of growth rate in acid buffers is due to the conversion of growth substance already in the plant from an inactive salt form to an active non-dissociated form.

TABLE 28

SAMPLE SERIES OF CURVATURES FROM INDIVIDUAL PLANTS

(Time of immersion; 2 hours)

Solution	Curvature of plant in degrees										Mean
pH 4.1	84	71	29	23	50	73	66	73	80	76	63 ± 7
pH 5.2	12	11	19	9	18	13	10	24	16	12	14.3 ± 1.5

TABLE 29

CHANGE OF CURVATURES WITH TIME

pH 4.1

(Curvatures in degrees)

Expt.	2 hr.	4 hr.	6 hr.	8 hr.
I	51	54	54	54
II	41	44	44	41
III	44	44	44	42

TABLE 30

EFFECT OF ACID AND NEUTRAL BUFFERS ON GROWTH OF EPIDERMAL STRIPS

Expt.	pH	Elongation in % during		No. strips
		1st hr.	2nd hr.	
I	4.1	0.4	0.2	4
II	4.1	0.3	0.0	4
III	7.2	0.4	0.2	4
IV	7.2	0.1	0.2	4
V°	4.1	7.0	3.1	6

°Plants with "epidermis removed".

TABLE 31

EFFECT OF ACID AND NEUTRAL BUFFERS ON GROWTH RATE OF PLANTS HAVING EPIDERMIS REMOVED FROM BOTH SIDES

Buffer	% Elongation per hour						No. plants
	1 hr.	2 hr.	3 hr.	4 hr.	5 hr.	6 hr.	
pH 4.1	6.9	3.1	1.6	1.0	0.6	0.5	18
pH 7.2	0.3	0.4	0.3	0.4	0.3	0.2	18

TABLE 32

ACID CURVATURES OF PLASMOLIZED COLEOPTILES°

Expt.	Curvatures in pH 4.1		Curvatures in pH 7.2	
	Plasmolized	Not plasmolized	Plasmolized	Not plasmolized
I	+3.2°	-74°	+0.2°	-4.0°
II	+4.0°	-68°	+3.3°	+2.0°

°These plants were young (coleoptiles 1 to 2 cm. tall) and therefore did not exhibit the plasmolysis curvatures discussed in this part.

TABLE 33

EFFECT OF INFILATRATION UPON THE GROWTH RATE OF COLEOPTILE SECTIONS IN BUFFER SOLUTIONS

	pH	Growth in % per hr. during						Plants
		1 hr.	2 hr.	3 hr.	4 hr.	5 hr.	6 hr.	
Infiltrated	4.1	5.8	3.2	1.5	1.1	0.8	0.3	12
Not "	4.1	3.2	3.1	2.6	2.0	1.9	1.3	12
Infiltrated	7.2	0.6	0.2	0.1	0.2	0.3	0.2	12
Not "	7.2	0.9	0.5	0.6	0.7	0.5	0.2	12

TABLE 34

EFFECT OF ACID UPON THE PLASTICITY OF COLEOPTILES

Expt.	Plants	Growth in pH 4.1	Growth in pH 7.2	Time in buffer hr.	Elastic curvature in degrees pH 4.1	Elastic curvature in degrees pH 7.2	Plastic Curvature in degrees pH 4.1	Plastic Curvature in degrees pH 7.2
I	11, 11	6.2	0.1	4	---	---	24.5	3.3
II	11, 11	6.9	0.1	2	6.1	5.0	25.7	4.3
III	10, 8	2.5	0.0	1.5	6.3	4.8	13.2	3.9
IV	12, 10	1.5	0.0	1	4.1	4.5	13.5	2.1
V	10, 9	1.6	0.0	1	5.5	4.7	16.6	2.7

TABLE 35

ACIDITY OF FAST GROWING AND SLOW GROWING SECTIONS OF COLEOPTILES

Quinhydrone electrode — Pot. with calomel half cell, volts

Expt.	"Tops"	"Bottoms"
I	0.109 0.104	0.116 0.108
II	0.100 0.099 0.094 0.094	0.101 0.097 0.097
III	0.088 0.085	0.087 0.091
IV	0.102 0.097	0.112 0.100
V	0.102 0.091 0.096	0.107 0.096 0.096
VI	0.093 0.099	0.094 0.100
Mean	0.096 ± 0.002	0.100 ± 0.002
$pH = \frac{-E + 0.454}{.0591}$	= 6.06±.03	5.99±.03

Glass electrode — Pot. with 1.07 HCL, volts

Expt.	"Tops"	"Bottoms"
I	0.287 —	0.279 0.281
II	0.287 0.287	0.284 0.279
pH	6.17	6.04

TABLE 36

pH OF SECTIONS OF *AVENA* COLEOPTILES IN 1% FRUCTOSE PLUS GROWTH SUBSTANCE AND IN DISTILLED H_2O.°

Expt. No.	Potential in volts: In growth substance	Potential in volts: In H_2O
I	0.090	0.094
	0.088	0.089
II	0.084	0.088
III	0.092	0.094
	0.090	0.094
IV	0.090	0.092
	0.089	0.091
Mean	0.089±.001	0.091±.001
pH	6.18±.02	6.14±.02

° Time in solutions = 4 hours

TABLE 37

EFFECT OF GROWTH SUBSTANCE UPON GROWTH RATES IN ACID AND NEUTRAL SOLUTIONS

Solution	Plants	Growth in % per hr. during 1 hr.	2 hr.	3 hr.
°g.s. in distilled H_2O	18	10.9	3.4	1.2
pH 4.1 buffer	18	6.9	3.1	1.6
g.s. in pH 4.1 buffer	18	12.0	4.2	0.6
pH 7.2 buffer	18	0.3	0.4	0.3
g.s. in pH 7.2 buffer	18	0.8	1.0	0.3

°growth substance concentration = 10 units/cc

TABLE 38

EXTRACTION OF GROWTH SUBSTANCE FROM LOWER PORTIONS OF COLEOPTILES WITH $HCCl_3$.

No. of plants	Vol. of $HCCl_3$	Vol. of final solution	Angle	Vol. of blocks	g.s./plant
45	5cc	0.25cc + 0.25cc 3% Agar	4.2°	$10mm^3$	4.7 units

TABLE 39

EFFECT OF PREVIOUS DECAPITATION UPON ACID CURVATURE
pH 4.1

Expt.	Plants decap. 2 hrs. before	Plants decap. immediately before.
I	59°	160°
II	82°	110°

TABLE 40

GROWTH RATE OF PLANTS WITHOUT EPIDERMIS

Solution	Plants	% growth per hr. during					
		1hr.	2 hr.	3 hr.	4 hr.	5 hr.	6 hr.
g.s. 10 u/cc	18	10.9	3.4	1.2	0.6	0.3	0.2
pH 4.1	18	6.9	3.1	1.6	1.0	0.6	0.5
H_2O	18	2.3	0.9	0.4	0.4	0.2	0.2
g.s. + HCN $8x10^{-4}N$	12	0.8	-1.3				
pH 4.1 + HCN $8x10^{-4}N$	12	0.7	-2.1				
g.s. + HCN $4x10^{-4}N$	12	3.2	0.2				
pH 4.1 + HCN $4x10^{-4}N$	12	3.9	-1.0				
g.s. + HCN $2x10^{-4}N$	12	6.5	2.0				
pH 4.1 + HCN $2x10^{-4}N$	12	5.4	0.5				

TABLE 41

PER CENT INHIBITION OF GROWTH SUBSTANCE AND ACID GROWTH BY HCN

% inhibition in first hour					
Solution	HCN strength	%	Solution	HCN strength	%
pH 4.1	$8x10^{-4}$	90	g.s.	$8x10^{-4}$	93
pH 4.1	$4x10^{-4}$	43	g.s.	$4x10^{-4}$	71
pH 4.1	$2x10^{-4}$	22	g.s.	$2x10^{-4}$	41

TABLE 42

VARIATION OF ACID CURVATURE WITH pH

Expt.	pH 2.5	3.4	4.1	4.8	5.2	5.7	6.4	7.2	7.9
1	70	56	37	15	—	9	6	1	2
2	88	83	46	28	19	10	7	3	2
3	101	80	76	21	15	18	7	4	2
4	77	74	58	28	23	11	5	2	4
5	107	99	75	41	21	11	7	1	2
Means	89	78	58	27	19	12	6.4	2.2	1.6
$\frac{4.25^\circ}{9^\circ}$	42	37	27	13	9	5.7	3	1	0.75

TABLE 43

INTERNAL pH OF COLEOPTILES AFTER ACID CURVATURES

pH of buffer	2.5	3.4	4.1	4.7	5.7	6.4	7.2	7.9
pH of plant	4.7	4.8	5.2	5.7	5.9	6.1	6.3	6.9
pH expected from curve	4.8^{5}	4.9^{5}	5.1^{5}	5.5^{5}	5.9	6.2	6.6	7.0

TABLE 44

CHANGE OF ACID CURVATURE WITH pH IN HELIANTHUS HYPOCOTYLS AND PISUM EPICOTYLS

	Plants	pH	4.1	4.8	5.2	5.8	6.4	7.2
				(Curvature in deg.)				
Pisum	6		77	44	22	17	7	---
Helianthus	6		111	50	27	22	9	3

PART VIII.

GROWTH AND RESPIRATION

A. Introduction

In Part VI it was shown that aerobic metabolism is intimately associated with elongation under the influence of growth substance. The present section is therefore concerned with the respiration of the coleoptile, particularly in its relation to elongation. This work was undertaken with the hope that elongation would exhibit a close correlation with some relatively accessible property of the respiration, for example with its magnitude or its respiratory quotient. It may be said at once, however, that this was not the case and that the work of the present section, while revealing several points of interest and defining more clearly the dependence of elongation upon respiration, has not resulted in any solution of the ultimate fashion in which respiration is essential to growth.

Since the subject of respiration is rather far removed from the subjects of the preceding parts, it will be necessary to make a short review of the literature. It has long been known that the growth of an aerobic plant depends upon the existence of its aerobic respiration. Before the end of the nineteenth century, Wieler, and others had demonstrated that growth of the higher plants in general ceases or is greatly diminished in air of less than 0.1 to 3 per cent oxygen. Pfeffer (1903) expressed these findings simply in the statement that respiration is a "formal necessity" of growth. Sachs held essentially the same view. Sachs (1870) as well as others also realized that there is a certain amount of correlation

between the intensity of the respiration of a given plant and the intensity of its growth processes. Thus the respiration of a dormant seed is low but that of the developing seedling is high. This consideration lead several investigators to determine the course of respiration over the "grand period" of growth. De Saussure (1833) measured the respiration of germinating seeds, but more extensive studies were made by Mayer (1876) and Rischawi (1876). They found that the rate of respiration passes through a grand period similar to that of growth rate. This observation has been abundantly confirmed in more recent years, for example by Stahlfelt (1926), Kidd, West, and Briggs (1921) and by Hafenrichter (1928) for the higher plants and by a large number of workers (see De Boer, 1928) for fungi.

It has been shown by many, beginning with Malpighi and Godlewski that very low oxygen pressures decrease not only growth but also respiration, and that pressures of oxygen somewhat higher than that prevailing in the atmosphere often affect the rates of both sets of processes (Jentys, 1888). Changes of temperature have been also found to bring about qualitatively similar changes in growth and in respiration. Pfeffer pointed out, however, that the cardinal points for growth are frequently different from those of the respiration of the same organism. For example, the rate of respiration often continues to increase after growth has passed through its optimum temperature. This is well shown in the recent and thorough work of Mack (1930) upon the growth and respiration of wheat. There cannot be then any direct correspondence between magnitude of respiration and magnitude of growth rate.

There have been few attempts to analyze further whatever correlation may exist between growth and respiration. Pfeffer has contented himself with designating respiration as a formal necessity of growth. Sachs took the view generally accepted that respiration is essential to growth in that it furnishes the necessary energy and pointed out that respiration would be able to produce as a by product the organic acids shown by De Vries (1879) to be essential for the maintenance of the turgor of the cell. It has even been suggested that the principal function of respiration is the production of osmotically active substances.

It has long been assumed, rather gratuitously, that the biosyntheses and the mechanical work of growth utilize an appreciable if not a considerable portion of the energy liberated by the respiration of the growing plant. This assumption is reflected in the statement commonly found in textbooks (cmp. Kostychev, "Plant Respiration") that respiration is essential for growth because of the energy required in the latter process. In recent years it has been demonstrated that the major portion if not all of the energy given off by respiration ultimately appears as heat. Since this is the case, it is clear that respiratory energy has not entered into synthetic processes except in so far as needed to furnish energy of activation (hence for the overcoming of "potential humps"). This latter energy will of course appear ultimately as heat. On the other hand, energy which appears as heat may, it is true, previously have done useful mechanical work. Consider for example the work of plastic stretching of an _Avena_ coleoptile. Such work goes into what may be considered as the overcoming of friction of the particles

of cell wall. However, work used up in overcoming friction appears as heat. We are hence unable to decide from calorimetry whether or not respiratory energy has done mechanical work of this nature.

The question of the energy of growth can be asked in at least two ways: a) in what form does the energy lost from the organism and its nutrients appear; and b) in what form does the energy of respiration appear? These two questions cannot be answered with complete satisfaction at present, but the nearest approach to the answers may be found in the recent work of Algera (1932). This investigator has constructed a compensation calorimeter of considerable precision and has measured the heat evolved at constant temperature by a culture of Aspergillus niger growing on glucose and ammonia. At the same time the CO_2 production of the culture was measured, and the initial and final heats of combustion of the medium and mycelium estimated. Only this one work will be considered here since technically it is superior to those of Molliard (1922), Terroine and Wurmser (1922) and Doyer (1915) to mention only a few of the investigations on this subject.

Question a) will be considered first. During six days the medium lost 8810 calories, and the mycelium increased by 5606 calories. Therefore 3204 calories remain to be accounted for. 3299 calories appeared as heat, an excess of 95 calories or 0.5 per cent of the total heat transfer, and within the experimental error. It is clear that no considerable portion of the energy lost by the medium has gone into any sort of physical energy of "cell organization", which is not recoverable by combustion.

The answer to the other question demands a knowledge of the respiratory quotient during the time of CO_2 evolution, but unfortunately this quantity was not determined by Molliard, Terroine and Wurmser, or by Algera. If the quotient is taken as that of complete glucose combustion, the usual procedure, one finds that 3518 calories were evolved by the respiration. This is impossible since only 3204 calories at most were lost from the nutrient and mycelium. Hence it is clear that the respiratory quotient must have a value above one and that each mol of CO_2 does not come from a complete combustion of one sixth mol of glucose but that a portion of the CO_2 comes from fermentation of some kind.. Tamiya (1932) has shown that the respiratory quotient of Aspergillus oryzae on glucose is over 1 and Puriewitsch found that the R.Q. of A. niger on glucose is in the neighborhood of 1.1. If the R.Q. were in this case about 1.1 the energy of respiration would almost exactly balance the heat evolved and hence the loss of energy of the nutrient and solution. It should be remarked that this failure to establish the R.Q. before calculation of the energy of respiration from evolved CO_2 may lead to serious error.

For example Doyer calculated that the heat released by respiration during six days of germination of wheat seedlings was about two times the energy lost from the seedling as determined by heat of combustion. Stiles and Leach (1933) have found large variations in the R.Q. of all types of seeds during the course of development and it is certainly unjustifiable to assume a quotient of unity as was done by Doyer.

Since the energy of respiration comes rather close to balancing the heat evolved, it is clear that no major portion of it is stored as chemical energy of the plant material formed. In fact,

Algera even calculates that the formation of protein from glucose and NH_3 yields energy. The energy involved in the synthesis of protein from amino acids (as in a seedling) may likewise be presumed to be small. The formation of cellulose, which composes 60 per cent of the mycelium of Aspergillus according to the analysis of Terroine, Troutmann, Bonnet and Jacquot (1925), is slightly endothermic, but the free energy of cellulose formation cannot be calculated without a better knowledge of the concentrations in the cell. Since however, the cellulose is continually removed from solution, conditions should favor synthesis and the energy required be small or none.

When permissible methods are used, it is found, then, that within experimental error all of the respiratory energy is liberated as heat. However, as already pointed out, it cannot be said what portion of this energy may not have performed some useful function in growth before its final appearance as heat. There is, however, no question of an appreciable portion of the energy being permanently deflected to the synthesis of new cell material. So far as mechanical work is concerned a calculation of the pdv work during elongation of the coleoptile amounts to at most 0.4 per cent of the respiratory energy°. It seems clear therefore that some relation other than one based primarily upon energy considerations must connect respiration with cell elongation.

° Footnote: Consider a section 4.5 mm. long. It will have a cross-sectional area of approximately 0.16 mm^2 (Table 12). In two hours it may elongate roughly 0.45 mm. (Table 53) in buffer of pH 4.1. The cells of such a section have an osmotic value of approximately 10 atmospheres. The pressure exerted upon the cell wall may be much less (Ursprung and Blum, 1924). Taking 10 atmospheres as a maximum value and assuming constant internal pressure, the pdv work

done during elongation is therefore

$$10 \times 1.01\times10^{6} \times 3.1 \times 0.45 \times 0.16 \times 1\times10^{-3} = 2.3\times10^{3} \text{ ergs}$$
$$= 0.54\times10^{-4} \text{ calories.}$$

During the same two hours 3.0 mm^3 of oxygen (measured at standard conditions of temperature and pressure) will have been consumed (Table 54). Therefore the mols of oxygen used have been

$$3.0 \times \frac{1}{2.24\times10^{7}} = 1.3\times10^{-7} \text{ mols } O_2. \text{ (Table 62)}$$

During the same two hours the respiratory quotient was 1.07. The assumption will be made that the extra CO_2 was from a fermentation yielding energy negligible in comparison with that yielded by the aerobic respiration. Therefore

$$1.3\times10^{-7} \times \frac{1}{6} = 0.22\times10^{-7} \text{ mols hexose have been burned.}$$

Taking the heat of combustion of glucose as a rough measure of the energy liberated in the cell,

$$680,000 \times 0.22\times10^{-7} = 0.15\times10^{-1} \text{ calories have been}$$

liberated. The ratio of pdv work done to energy liberated is therefore

$$\frac{0.54\times10^{-4}}{1.5\times10^{-2}} = 0.36\times10^{-2}, \text{ or 0.4 per cent.}$$

In practically all of the works which have been mentioned thus far, "growth" has consisted of a complex of cell division, cell elongation, and cell differentiation. Either the growth of the entire plant, or in a few cases of the isolated root or stem apex has been considered. Another common failing is shown in the experiments of Mack (1930) in which the growth of the wheat "shoot" (presumably given by the length of the coleoptile) was compared with the respiration of the entire seedling. It is clear that a closer investigation of the relation of growth to respiration must, if possible a) differentiate between the different stages of growth, b) consider the two

processes in the same organ, or if possible in the same tissue. It is with these considerations in mind that the present study of the growth and respiration of the Avena coleoptile has been undertaken. The observable growth of this coleoptile, as already pointed out, proceeds almost entirely by cell elongation.

The present work is divided into two parts, 1) the distribution of growth and respiration both over the grand period of the coleoptile and in the coleoptile itself at the stage at which it is in general used for experimental studies on growth and tropisms; 2) a more particular study of the relation of the two processes in the most rapidly growing region of the coleoptile.

b. Methods. The measurements of respiration were carried out according to the manometric methods of Warburg (1926). The vessels which were used consisted of two sets of six each. One set, having volumes in the neighborhood of 16 cc., possessed central alkali wells and side receptacles for reagents to be added to the respiring tissues during the course of an experiment. The other set of vessels possessed gas volumes of about 5 cc. and could hence be used for the measurements of smaller gas exchanges than was possible with the first set. Unless otherwise stated, all experiments upon respiration were carried out in a thermostat at 25° C. The usual shaking device (Warburg, 1926) was used for agitation of the vessel contents during the experiments. All of the manipulations were carried out under red light in order to avoid possible phototropic stimulation, altho it is true that no significant effects of small amounts of ordinary light upon the rate of respiration could be found.

It is necessary to have the pieces of tissue small enough to insure that the rate of diffusion of oxygen into them does not limit the rate of oxygen uptake. Genevois (1927) has calculated that plant tissues under conditions very similar to those used here may be 0.4 mm. thick before the rate of oxygen diffusion becomes limiting. The tissue of the coleoptile is not over 0.4 mm. thick since this organ is hollow. It was found, however, that the respiration of the leaf, normally inside the coleoptile, is much accelerated if this leaf be removed and given free access to oxygen.

The growing and respiring tissues which were used were of course submitted to some injury as they were cut from the coleoptile. That a moderate amount of cutting has little influence upon the respiration of plant tissues has been shown by Stahlfelt (1926b) and by Genevois (1927). Table 45 shows that the respiration of these

(Insert Table 45)

sections is only decreased 5 per cent by a four fold increase in the number of cut surfaces and that a doubling of the number of cut surfaces has little effect. It is safe to conclude that the measured rate of oxygen uptake is a fair estimate of the oxygen uptake of the same section in the intact plant.

As with other properties of the coleoptile, the respiration rate varies from day to day. Table 54 gives a good idea of the extent of this variation. One reason for this undoubtedly lies in slight differences in the age of the sections used. Other governing factors may lie in the pretreatment of the seedlings or in the unknown factors causing variation of the growth substance activity from day to day (Kögl, 1933b). The duplicate samples from plants of the same day are

much more comparable as shown in Table 46.

(Insert Table 46)

Two growth substance preparations were used. The "crude growth substance" had an activity of about $2x10^{-6}$ mgs per plant unit, while the "purified growth substance" had an activity of about $3x10^{-7}$ mgs. per plant unit.

Unless otherwise stated the first three 4.5 mm. sections below the 5 mm. tip of coleoptiles 2.5 to 3 cm. tall were used. These will be known hereafter as standard sections.

c. The Coleoptile as a Whole

Elongation and Respiration During the Grand Period.

It is in the first place desirable to know if the changes in respiration during the grand period of coleoptile growth give any support to the view that there is an intimate connection between cell elongation and oxygen consumption. The grand curve of growth of the *Avena* coleoptile has been determined many times for example by Rothert, (1896), Sierp (1918), and by Konigsberger (1922). The changes of coleoptile oxygen consumption during this period have not been determined, but such determinations have been made upon other plants. Mayer (1875) and Rischawi (1876) found that the grand curve of respiration of germinating seeds was "similar throughout" to the grand curve of growth. More recently Kidd, West, and Briggs (1921) have shown that in *Helianthus* the respiration per unit dry weight follows closely the increase in dry weight per unit dry weight. Hafenrichter (1928) on the other hand found that in the soy bean, the maximum rate of CO_2 evolution per plant occurs earlier than the maximum rate of shoot elongation. This author however compared the respiration of the entire plant with the growth of the shoot alone, which is, as already pointed

out, not permissable.

Experimental. 2700 seeds were hulled and soaked for 12 hours. At the end of this time a rigid selection for those nearly in the same stage was made and the selected seeds placed upon moist paper to germinate. At the expiration of 24 hours, another selection was made and the selected seeds planted. Samples of plants were taken each 12 hours, and their lengths measured. Each of these samples was then suspended in 3 cc. of buffer, pH 4.1, placed in a manometer vessel, and its respiration rate measured over half hour intervals for 2 hours. At the end of this time the dry weights of two of the samples were determined after drying at 90° C. for four hours. The nitrogen contents of the other two samples were estimated by the micro-kjeldahl method. The weight of nitrogen determined in this way, gives, when multiplied by 6.1, an approximate value for the weight of protein present in the plant.

It is impracticable to separate the coleoptile from the very young embryo. Therefore at the ages of 12, 24, 36, and 48 hours the entire embryo with the exception of the scutellum was dissected out and used. At 48 hours the entire embryo was compared with the shoot alone. The entire shoot was then used at 48, 60, and 72 hours. At 72 hours the entire shoot was compared with the coleoptile alone, and the coleoptile alone used thereafter. In all cases the duplicate determinations of respiration, dry weight, and nitrogen agreed quite satisfactorily. Table 47 gives a summary of the data obtained.

(Insert Table 47).

Between the ages of 36 and 48 hours the root breaks from the coleorhiza and elongates rapidly. This accounts for the large increase in length, and apparently, for the large increase in res-

piration of the entire embryo at 48 hours. Between 96 and 108 hours the third leaf (Avery, 1930) breaks through the coleoptile. At this time the rate of elongation of the coleoptile falls swiftly to 0. From the data of Table 47 it is possible to calculate the approximate values of the respiration rate, dry weight, and protein content of the coleoptile alone over the entire grand period. The values of each quantity before 72 hours give obviously maximum figures, above which those of the coleoptile cannot be. At 72 hours the coleoptile possesses 82.5 per cent of the entire shoot respiration, 85.5 per cent of the entire shoot length, 50.5 per cent of the dry weight, and 46.5 per cent of the total protein. One can assume that as a first approximation these values hold over the course of development of the shoot before 72 hours. At 48 hours the relation between shoot and entire embryo is complicated by the presence of roots. Between 36 and 48 hours the respiration per unit dry weight and per unit protein increases markedly, indicating that the respiration of young roots is intense. However, it will be noticed that the respiration per unit dry weight of the 48 hour shoot is almost the same as that of the 36 hour embryo. Since the ratio of shoot dry weight to embryo dry weight in the 36 hour embryo is known, the approximate values of the shoot respiration may be calculated for 36, 24, and 12 hours. It should be emphasized that the calculation of these approximate values is permissible only since in any event the quantities concerned are rather small in comparison with their magnitudes later in the grand curve. Table 48 gives the summarized data for the

(Insert Table 48)

coleoptile alone. The rate of oxygen consumption goes through a

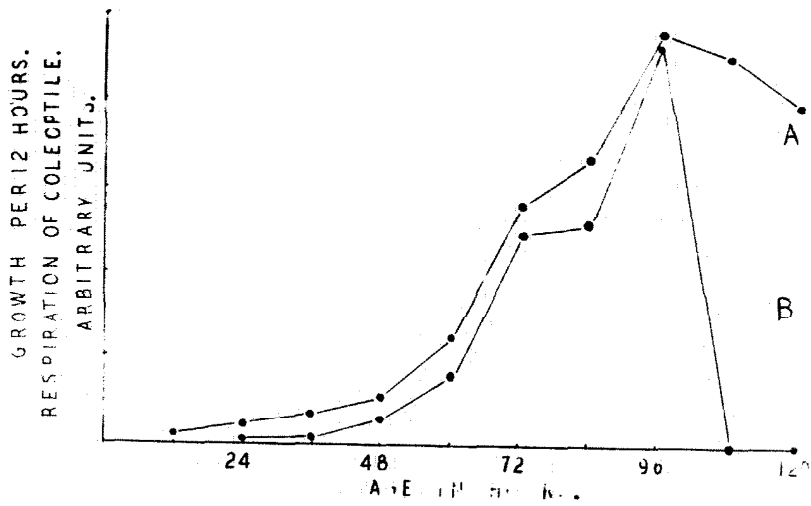

Figure 10.Growth and Respiration During the Grand Period of the Coleoptile. Curve A, Rate of Respiration at end of each 12 Hour Period. Curve B, Total Growth During Each 12 Hour Period.

very clear grand period, increasing steadily until growth of the coleoptile ceases, and then falling off. Rate of oxygen consumption is plotted together with total elongation per period, against time in Figure X. The parallelism between the two curves is very marked. Every change of slope of one curve is accompanied by a change in slope of the same sign in the other. At the time at which the leaf penetrates the tip of the coleoptile and growth of the latter falls to zero, the respiration falls, altho only 6 per cent. At most then 6 per cent of the respiration might be directly involved in growth.

The comparison of total coleoptile respiration with total coleoptile elongation is only one of the ways in which the data may be considered. If one considers, however, the respiration per unit dry weight, it may be seen that with the exception of the two earliest periods, there is a steady falling off with increase in the age of the plant, i.e. the maximum of the grand period occurs very early, in agreement with the findings of Kidd, West, and Briggs (1921) and Stalfelt (1926). One might compare the respiration per unit dry weight with the total elongation or with the growth rate. In either case the maximum rate of respiration is earlier than the maximum growth and the parallelism of Figure X is not apparent. Table 47 shows that the respiration per unit protein is remarkably constant, and falls off only slightly with age, whereas the respiration per unit dry weight falls off much more swiftly. This points to the fact that in general, comparisons of respiratory rate should be made upon the basis of the living matter present and not upon the dry weight. This was pointed out long ago by Palladin (1926).

Between 48 hours and 96 hours the coleoptile increases its length by 20 times. Despite this fact the dry weight per unit length

remains almost constant and the percentage of protein falls only to one half of its original value. There has taken place then not only synthesis of cell wall but also of protoplasm. The normal growth of the coleoptile consists not only of vacuolation and water uptake by protoplasm already present, but is accompanied under these conditions by protoplasm formation. In fact, inspection of Table 47 reveals that under these conditions elongation, respiration, dry weight, and protein parallel one another rather closely. This is, as already pointed out, probably related to the fact that the temperature used is near the optimum for the growth of Avena, since at this temperature one would expect the various processes to fit rather accurately together. Another point to which attention should be directed is the continued increase of dry weight after cessation of coleoptile growth. This is in accord with the fact that coleoptiles become much stiffer with time after cessation of growth.

Under the present conditions, then, the rate of total respiration of the coleoptile parallels qualitatively the amount of total elongation. This may indicate that the two processes are either directly connected or that they are effected in the same way by a common factor. It may, however, only indicate that protoplasm is formed during elongation and that respiration per unit protoplasm is rather constant.

c. Distribution of Growth and Respiration in the Coleoptile

The preceding section has shown that during the grand period of the coleoptile, growth is paralleled qualitatively by the grand period of coleoptile respiration, altho there is no quantitative relation.

This section will deal with the distribution of growth and respiration over the length of the coleoptile. The comparison in this case is between sections of physiologically different age instead of between coleoptiles of different age as in the previous section.

The growth distribution in the *Avena* coleoptile was first determined by Rothert (1893) but it has been more extensively studied by Went (1928). In plants of the ordinary experimental age (about 3 cm. tall) the growth rate rises from 0 at the extreme apex to a maximum about 10 mm. lower. From this point to the node of the coleoptile insertion the growth rate falls steadily. Fig. XI gives an example of this distribution taken from Went.

Experimental. Plants of as nearly the same height as possible were selected, tips exactly 5 mm. long removed, and the leaves extracted in the usual manner. The balance of the coleoptile was then divided into two six mm. zones and the base or remainder. The respiration rate of each set of sections was determined separately. The dry weights and microkjeldahl nitrogens of similar lots of sections were also determined.

Results. Table 49 gives the distribution of respiration in three typical experiments. Altho there are minor variations in each experiment, in the mean the respiration is fairly constant over

(Insert Table 49)

the entire coleoptile length. The reason for this constancy of respiration through the coleoptile could be explained as due to the synthesis of protoplasm during elongation as discussed in the preceding section. Table 50 shows that in fact there is more protein

(Insert Table 50)

per mm. in the base than in the top, altho due to the increase of dry weight toward the bottom, the per cent of protein is somewhat higher in the tip. The respiration per unit protein falls offcontinuously from the tip to the base, the values being 24.6, 22.3, 19.1, and 17.7 respectively. This is just as was found in the previous section for the grand period of respiration, where the respiration per unit protein decreased continuously with the age of the coleoptile during the later stages, and is an example of the fact that the cells of the tip are "physiologically younger" than those of the base. This decrease of respiration per unit protein might in fact be added to the manifestations of "aging" summarized by DuBuy and Nuernbergk (1932).

The distribution of growth rate in the coleoptile was interpreted by Went as due to the interaction of two factors, a) growth substance, present in excess in the tip and limiting in the base, b) "Zellstreckungmaterial" (including all factors operating from the base) present in excess in the base and limiting in the tip. It has been shown above that neither in the grand period nor in the distribution over the coleoptile is there a quantitative correlation between respiration per mm. and growth per mm. This makes it impossible that respiration or respirable material is the second factor of Went, For example, if there is sufficient respiration to allow of the growth rate of the fastest growing zone, then since the rate of respiration is the same in the tip or slower growing zone, respiration cannot there be limiting. Table 49 shows that the increase of respiration by carbohydrate (fructose) is small in every section and hence it is probable that ordinary nutrients are not the second

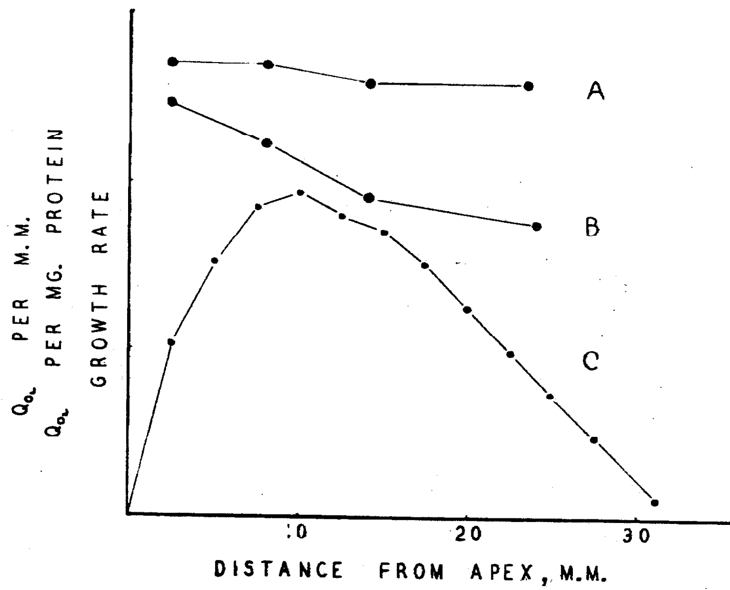

Figure 11. Distribution of Growth and of Respiration in the Coleoptile. Curve A, Rate of Respiration per m.m. Curve B, Rate of Respiration per mg. Protein. Curve C, Growth Rate, (After Went. 1928).

factor. It is true however, that of the small increases in respiration upon the addition of fructose, that of the tip was greatest and that of the base least. In conclusion it should be pointed out that the two factor interpretation of growth distribution is in reality somewhat oversimplified, since it has been demonstrated in Part VI that the lower zones give a lower response to a given concentration of growth substance than do those higher up.

d. The Rapidly Growing Zone

Changes of growth and respiration with time. The changes of growth rate and respiration rate of coleoptile sections with time have been determined many times, always with approximately the same result. Figure XII shows the course in a typical experiment in which coleoptile sections were suspended in buffer of pH 4.1 made up to 10 units of growth substance per cc. (Here as later unless otherwise stated the purified growth substance of activity $3x10^{-7}$ mgs. per plant unit was used). The growth rate falls steadily until in the eighth hour it has fallen to 2 per cent of its original value. The rate of respiration, on the other hand, after an initial period of sinking remains rather constant at about 82 to 84 per cent of its initial value. It is, however, during the most rapid fall of growth rate that the fall of respiration takes place. From this it is clear that if there is any quantitative relation between growth and respiration, then at most about 17 per cent of the latter is essential to the former, since 82-83 per cent of the respiration can go on with very little accompanying growth.

The fact that the respiration is well maintained for several hours shows that there is no question here of deficit of nutrients.

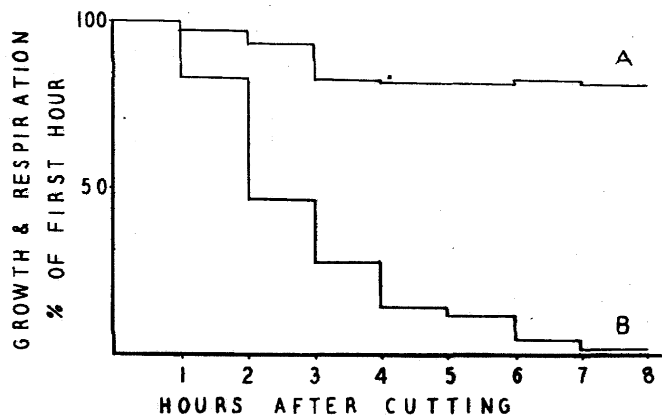

Figure 12, Changes in the Growth Rate and the Respiration Rate of Coleoptile Sections with Time. Curve A, Respiration Rate. Curve B, Growth Rate.

This is in accord with the fact shown in Table 49 that the addition of fructose to the medium has but little effect upon the respiration.

Effect of cyanide upon growth and respiration. It hasbeen previously shown that growth and respiration are inhibited by cyanide to about the same extent. A quantitative examination of the inhibition has now been made to determine more precisely the relation.

Sections were suspended in buffer of pH 4.1 which had been made up to a concentration of 10 units per cc. in growth substance. The desired quantity of freshly prepared 0.1 molal HCN was then added. The growth rates of a portion of the sections were measured with the horizontal microscope, and the respiration rates of the remainder determined in the usual fashion. The inhibition of growth is expressed in per cent of the growth of controls not receiving HCN. This is also true of all of the respiration inhibitions with the exception of those using $5x10^{-5}$ molal HCN. Here upon account of the small inhibition it was found more satisfactory to measure the same sample before and after the addition of HCN. It should be noticed that in these experiments HCN solutions are used in vessels containing KOH in the alkali wells. This causes a falling off of the inhibiton with time as the HCN goes over into the KOH. The inhibition was found to be rather constant over the first hour, and in these experiments only the first hour is considered, since even during the second hour the higher concentrations proved toxic. (Concerning the use of HCN with vessels containing alkali see also Emerson, 1928).

Tables 51 and 52 gives the results of several experiments. In general the inhibiton of growth is rather reproducible. The

(Insert Tables 51 & 52)

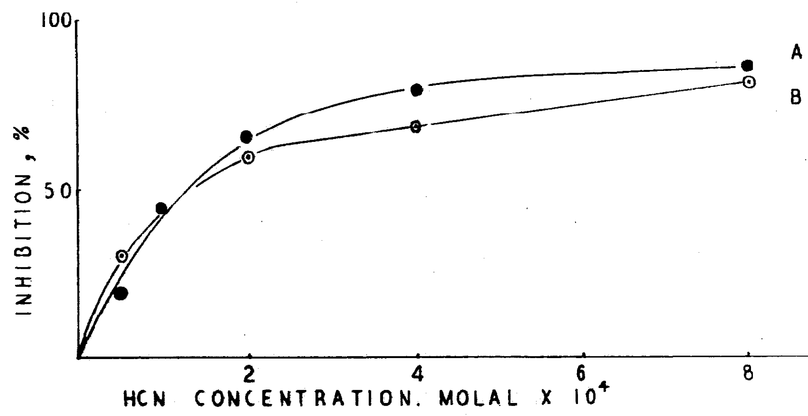

Figure 13. Inhibition of Growth and of Respiration by HCN. Curve A, Inhibition of Respiration Rate. Curve B, Inhibition of Growth Rate.

inhibition of respiration is much more variable especially in the lower concentrations as is shown by the large errors. Figure XIII presents the data of Tables 51 and 52 graphically. The two inhibitions parallel one another rather closely altho there is a suggestion of smaller HCN sensitivity of respiration at $5x10^{-5}$ molal and of smaller HCN sensitivity of growth at $4x10^{-4}$ molal. It seems fair to conclude that a given reduction of respiration by HCN is accompanied by a comparable reduction in the rate of elongation.

Action of growth substance upon growth and upon respiration. The activity of growth substance in promoting cell elongation depends principally upon the undissociated acid present (Part VII). However, the pH of the cell is such (6.1) that only 4 to 5 per cent of the growth substance which it contains is present in the active form. If the cell acidity is increased by placing the section in an acid buffer solution, more of the cell's own growth substance becomes active and its rate of elongation is increased even without the addition of more growth substance. If the sections be placed in a neutral or basic solution, on the other hand, the cell acidity becomes less than normal and more of its growth substance is changed to the inactive form with the result that the rate of elongation of the section is decreased. Table 53 gives a summary of the growth rates

(Insert Table 53)

under different conditions of acidity and of growth substance. (The sections of Table 53 were not infiltrated but merely placed in the different solutions). The purpose of the present section is to compare the rates of respiration under these different conditions.

Table 54 presents the results of 11 experiments which demonstrate that there is no appreciable difference in the rate of oxygen uptake at pH 4.1 and 7.2 despite the different growth

(Insert Table 54)

rates in the two cases. This is in agreement with Gustavson (1920) who found no effect of pH upon the rate of mould respiration. In a preceding section the maximum portion of the respiration which might be directly due to elongation was shown to be less than 6 per cent. In another section this portion was found to be less than 16-17 per cent. The present experiment shows, however, that if such a respiration exists, its oxygen consumption is negligible in comparison with the total.

In an earlier paper (Bonner, 1933) it was shown that certain growth substance preparations cause an increase in the rate of respiration. It was suggested that altho this might prove to be a property of the hormone itself, it might on the other hand be due to associated impurities. It can be shown by the use of a number of different growth substance preparations that the latter supposition is the correct one. For example, consider the two preparations, "bulk CCl_4 extract", activity $2x10^{-6}$ mgs. per plant unit, and "brucine filtrate", activity $3x10^{-7}$ mgs. per plant unit. Table 55 shows that while the former gives a

(Insert Table 55)

considerable increase in oxygen uptake, the latter has no effect. The stimulating effect of the crude growth substance preparation is probably due to a specific stimulating substance and not to the

nutritive action of associated organic material for the following reasons:

a) it is shown that fructose which should also exhibit a nutritive action has but little effect on respiration;

b) in the above mentioned paper (Bonner, 1933) it was shown that the stimulating action of the crude extract is destroyed by oxidation with H_2O_2.

It has been found by Schwabe (1932) and by Burge, Wickshire, and Estes (1928), that amino acids in small amounts cause considerable increases in the respiration of various plants, as Elodea, Fontinalis, Vallisneria, and Spirogyra. Amino acids cannot be present in the growth substance preparations which have been extracted with ether. However, the effects of two amino acids in concentrations near the optimum given by Schwabe were investigated and the results are given in Table 56, together with the effect of fructose for comparison. Under the present conditions alanine has no effect and histidine is slightly toxic.

(Insert Table 56)

Effect of temperature on the two processes. Another external factor whose effects upon the two processes might be compared is temperature. The literature concerning the effect of temperature upon growth as well as that concerning respiration is tremendous and only a few of the references will be made here.

De Candolle (1835) first determined the cardinal temperatures for thegrowth of a variety of seedlings. This type of work was extended by Sachs (1879), Köppen (1870) and others. More recently interest has centered upon the effect of temperature upon the actual growth rate. Leitch (1916) found that

the rate of root growth in Pisum possesses a Q_{10} of 1.9 to 2.0 between 10° C. and 25° C. Somewhat higher values were found by Lehenbauer (1914) for Zea mais. Talma (1918) obtained results similar to those of Leitch for roots of Lepidium. Balls (1908) found that between 15° and 30° C. the rate of growth of fungal hyphae has a Q_{10} of 2 to 3. On the other hand there are examples of lower temperature coefficients for elongation. Hamada (1930) gives data from which it may be calculated that the Q_{10} of elongation of the normal Avena coleoptile between 15° and 25° C. is 1.7. The Q_{10} is still lower if longer periods are considered since coleoptiles grow actually longer at 15° than at 25°. Mack (1930) has shown that the Q_{10} (at the oxygen pressure of ordinary air) of Triticum shoots between 15° and 25° C. is about 1.5 and that the Q_{15} between 10° and 25° is 2.4. It is also of interest that the Q_{10} of the reaction time of phototropic response in the Avena coleoptile has been found by M. DeVries (1914) to be about 1.5.

The earlier literature on the effect of temperature upon respiration has been summarized by Pfeffer (1900) in the statement "respiration exhibits no distinct optimum lying within limits of temperature which can be endured for prolonged periods", and contrasts this with growth which in general shows a distinct and relatively low optimum. Kuyper (1910) investigated the matter in some detail, and found that "Van't Hoff's rule" held over the range of 0° to 20° C. for the respiration of Pisum and Triticum. Measured over short periods, (one hour) respiration increased steadily up to 45° C., or far over the optimum growth temperature. Measured over longer periods the respiration showed an optimum at about 35° C., due to progressively greater

injury of the plant at higher temperatures. A careful study of the effect of temperature upon the respiration of Vicia faba has been made by Navez (1929), who found Q_{10} values of 2.3 and 2.7 for the intervals 15° to 25° C., and 10° to 20° C. respectively and these values for the Q_{10} are those usually found for respiration.

Concerning the relative effects of temperature changes on the two processes of growth and respiration, it has already been mentioned that Pfeffer pointed out the general lack of correspondence between the cardinal points of the two processes, and suggested that at the higher temperatures the two might compete for nutritive substance. The data of Mack (1930) show an increase in temperature is more effective in increasing the respiration than in increasing the growth rate of the shoots of wheat. Kidd, West, and Briggs (1921) believe that their data point to a lower temperature coefficient of growth than of respiration. However, their measure was that of relative increase in dry weight and is hence not comparable with the other studies mentioned above.

The work of Hamada upon Avena seedlings and that of Mack on Triticum has shown that the Q_{10} of elongation of the coleoptile between 10° and 25° C. is distinctly below 2, while the Q_{10} of respiration is markedly higher. It will now be shown that this is also the case for coleoptile sections growing in growth substance solutions.

The growth and respiration of comparable lots of sections at two temperatures were compared, using in every case the plants of the same day. Since all that was desired was a comparison of the effect of lowered temperature upon the two processes, only two temperatures were used, namely 25° and 10° C. Two thermostats were

used at the same time, the one being kept at 10° C. by the cooling unit of an electric refrigerator; and the other being maintained at 25° C. Comparable sections were suspended in buffer of pH 4.1 made up to 10 units of growth substance per cc. One half of the sections were then placed in vessels in the 10° C. thermostat, and the other half in vessels at 25° C. Measurements of the pressure decrease were made each half hour, and at the end of the desired period, the sections were removed and measured under the binocular microscope with a calibrated eye piece micrometer. It is to be noted that in these experiments the sections were 3.5 mm. long whereas those of the previous experiments were 4.5 mm. long.

Table 57 presents a few typical results. As longer periods are considered, the ratios of the amounts of elongation and of the total amounts of respiration at the two temperatures, both decrease, altho the former decreases much more. In the case of respiration it may be seen that this is due to the fact that the respiration at 25° sinks with time whereas that at 10° actually increases a small amount. In the case of growth the ratio decreases because elongation continues for a longer time at 10°, i.e. elongation continues at 10° after it has almost ceased at 25°. Measurements of the hourly growth rate showed that in fact after the fourth hour the growth at 10° is actually faster than that at 25°. The Q_{15} of the first hour was also found to be somewhat higher than that of the first two hours, but in no case was it over 2.6.

(Insert Table 57)

Table 58 gives the summary of a number of 2 hour determinations of respiration rate and elongation rate at the two temperatures, together with the Q_{10}'s calculated with the aid of the assumption that the change of rate with temperature is linear over the range considered. The work of Navez (loc. cit.) and of Kuyper (loc.cit) shows that this assumption is justified in the case of respiration at least, particularly since an aproximate value is all that is desired for comparison with the work of other investigators. The Q_{10} of respiration over this range is 2.6 and hence well within the range found by Navez and by Kuyper. The Q_{10} of elongation is however 1.7 and thus similar to the values found by Mack and by Hamada. It is of interest that Heyn and VanOverbeek obtained a Q_{10} of about 1.6 for the rate of plastic bending of Avena coleoptiles after growth substance entry from agar blocks.

Characteristics of Growth and Non-Growth Respiration.

(a) The HCN sensitivity. It was next desired to find out if the respiration of a coleoptile section is qualitatively changed in any respect by the intensity of its growth. The sensitivity to HCN was the first characteristic of the respiration which was examined. It has been found by Genevois (1927) that the sensitivity of the respiration of the stems of Lathyrus to KCN decreases as the stems become older. At the same time the respiration per unit dry weight decreases in a manner similarto that described earlier for coleoptiles. The respiration of a section from a 108 hour old coleoptile will, of course, be less than that of an 84 hour section since as shown in Table 48 the respiration per unit length is less. Table 59 shows that the

(Insert Table 59)

sensitivity to cyanide is also less. Sections which have ceased growth due to age have then a smaller proportion of their respiration inhibited by a given concentration of HCN than do younger, actively growing sections.

Sections respire at the same rate in solutions of pH 4 as in solutions of pH 7, altho the growth rates are different. This offers therefore an opportunity to compare the sensitivity of sections to HCN, the sections having the same rate of respiration but different growth rates. Cyanide inhibition is proportional to the amount of free HCN present in a solution, probably because only HCN enters the cell. Therefore in comparing the inhibition at two different pH's it is important to consider only the fraction of the total cyanide present as free HCN in the two cases. In all of the present experiments cyanide was added as freshly made up HCN. The pK of HCN is, at

25° C., 9.14. Therefore in a solution buffered at pH 7, less than one per cent of the cyanide is present as CN^- ion. At both pH 4 and pH 7, then, the inhibition will depend upon practically all of the added cyanide. Table 60 presents the results of 5 experiments.

(Insert Table 60)

At the lower HCN concentration, the inhibition exhibits the same variability previously noted. At the higher concentration the inhibition is remarkably constant from one experiment to the next. At both of the HCN concentrations there is a quite significant difference between the sensitivities in the two buffer solutions. Moreover it is interesting to note that the per cent inhibition at pH 7 is of the same order as that shown in Table 59 for sections at pH 4 whose growth has ceased due to age.

The cyanide sensitivity of elongation is practically the same at pH 7 as at pH 4 (Table 61). This is an added confirmation

(Insert Table 61)

of the supposition that the elongation process is essentially the same under the two conditions. The cyanide sensitivity of elongation lies between that of respiration at pH 4 and that of respiration at pH 7, altho somewhat closer to the former.

From this experiment it might be said that respiration at pH 4 does differ somewhat from that at pH 7 and that the former kind of respiration is accompanied by the faster elongation. Respiration at pH 4 also resembles elongation somewhat more closely in this one characteristic than does respiration at pH 7.

(b) The Respiratory Quotient. The preceding section has shown that the respirations of fast growing and slow growing sections differ in at least one respect, namely in HCN sensitivity. Another characteristic property of a particular type of metabolism is its respiratory quotient. The value of this quantity in fast and slow growing sections was therefore studied.

Most of the determinations of the respiratory quotient of plants since the days of Bonnier and Mangin (1884) have concerned themselves with germinating seeds and changes in the respiratory quotient due to changes in the food reserve. In relatively few cases have the respiratory quotient of the embryo alone been examined. That there may be large differences between the quotient of the entire seedling and that of the young plant alone is well shown by the work of Murlin (1933), who found that while the respiratory quotient of the entire plant and seed (Ricinus) was 0.3 to 0.4, thatof the young plant alone was 0.8 to 1.1.

The quotients were determined according to the method described by Warburg (1928). The method consists essentially in measurements of a) the gas which may be driven out of the tissue and solution by acid at the beginning of the experiment ("preformed CO_2"); b) the net gas exchange and gas drivenoff by acid at the end of the experiment; c) the oxygen consumption during the experiment.

Coleoptile sections were suspended in the solution to be used and placed in the vessels having side arms. In vessels for the determination of a) and b) no alkali was placed in the well, but 0.4 cc of 4 molal H_2SO_4 was placed in the side arm. At thedesired moment this acid could be tipped into the main body of the vessel and the change in pressure noted after the reestablishment of temperature

equilibrium. There is a small volume change of the solution upon addition of the acid, but this was corrected for. For the determination of c vessel containing KOH in the alkali well and acid in the side arm was used. This acid was tipped in at the same time as that for the determination of b, in order to measure the oxygen uptake during death of the sections as well as during the main body of the experiment.

The determination of the respiratory quotients revealed a peculiar fact which has however, so far as can be seen at present, no relation to the action of growth substance and whose significance has not yet been studied further. The respiratory quotient is markedly higher for sections in acid buffer than for sections in neutral buffer. Table 62 shows the results of a series of experiments, each lasting two hours. In buffer of pH 4 the quotients are very constantly more than 1. In buffer of pH 7 they are more irregular but are markedly below 1. It must be emphasized that the fact that during the main body of the experiment most of the CO_2 is driven off by the buffer of pH 4 and a portion of it is retained by the buffer of pH 7 does not affect directly the determination of the quotient since in both cases all of the CO_2 is ultimately driven off with excess acid. The buffer was only 0.025 molal whereas the final acidity of the solution was 0.85 molal. In order to make clear the method of calculation as well as to illustrate the lack of effect of growth substance upon the respiratory quotient, Table 63 gives in full the data of three typical six hour experiments. The total elongation of comparable sections for the same period is included for comparison. In the unbuffered fructose solution the quotient is nearly unity as would be expected. Growth substance has no effect. In buffer of pH 4

the quotient is very high. Growth substance, altho increasing the growth has no effect upon the quotient.

Strugger (1932, 1933) has suggested that fermentation favors growth by cell elongation. The high quotient in buffer of pH 4 might indeed be construed as due to a sort of a fermentation. However, such a high quotient is not essential to growth as is shown in Table 63.

(Insert Tables 62 & 63)

Moreover, if fermentation were favorable to growth one might expect that anaerobic conditions should stimulate growth or that HCN in suitable amounts should, by inhibition of the "Pasteur reaction" increase the growth rate. An increase of growth rate by either anaerobic conditions or HCN has never been observed in the course of these investigations.

The action of growth substance. Altho respiration is necessary for elongation, the decrease of elongation, for example, by decrease of temperature or increase of pH, is not proportionally the same as the decrease of respiration. This could be due to a fusion of the characteristics of several consecutive component processes, only one of which is a respiratory process, into the characteristics of the elongation process as a whole. An attempt was therefore made to separate these component processes from one another.

The steps involved in the bringing about of elongation by the application of growth substance may be divided into those of uptake, subsequent action, and actual elongation. It has been shown in Part VI that respiration is necessary for the passage of growth substance into a coleoptile section. Nevertheless the Q_{10} of this process was shown in a previous section to be approximately 1. It

is likely that the rate of passage of growth substance into the coleoptile is limited not by the rate of transport within the section, which has the high Q_{10} of 2.6 (Van der Wey, 1932) but by diffusion across the cut surface, which should have a low Q_{10}. If the temperature coefficient of entry be measured by subsequent elongation in water at 25° (Table 64) or by plasticity at 25° (Table 64) it is

(Insert Table 64)

also found to be one. However, the elongation of sections in solution is not limited by the rate of entry of growth substance as was shown in Part VI where it was demonstrated that the growth rate of sections in growth substance solution is greater than that of sections already full of growth substance from previous treatment with growth substance containing agar blocks.

It has been assumed by Heyn (loc. cit.) that if growth substance agar blocks are placed upon plants without water supply, an "action" of growth substance takes place which results in increased plasticity and, if the plant is then placed in water, an increased growth rate. That some small amount of such "action" does take place is possible. A large part of this "action" must however take place later for the following reasons;

a) Table 65 shows that if sections without water are allowed to take up growth substance, the subsequent elongation is still greatly

(Insert Table 65)

influenced by pH of the solution. These sections are already full of growth substance, which if action takes place apart from elongation, should have acted. Nevertheless elongation depends upon the presence

of undissociated growth substance, which has been shown (Part VII) to be essential for elongation as a whole.

b) Growth substance action has been shown (Part IV) to be of necessity indirect, i.e. chemical and would then be likely to have a Q_{10} greater than 1. The Q_{10} of growth substance effect prior to actual bending or elongation is, however, 1, i.e. that of growth substance entry

c) The Q_{10} and cyanide sensitivity of elongation after the entry of growth substance from agar blocks are similar to the Q_{10} and cyanide sensitivity of elongation as a whole (Tables 66 and 58).

(Insert Table 66)

Therefore the limiting process of elongation takes place after block action, i.e. must take place during the actual deformation of the wall.

d) That the actual increase of wall plasticity takes place largely during wall deformation is not in conflict with the experiments of Heyn. He found that coleoptiles without water and with or without growth substance showed the same extension under tension if they were immediately plasmolized. Increases in wall plasticity depended upon the turgidity of the plants.

One is forced then to the conclusion that a continuous action of growth substance is necessary for increases in wall plasticity and hence for elongation. This increase in plasticity is not separable from the actual extension process which it makes possible, at least so far as the present methods are concerned. Since this is the case, it is impossible to determine, for example, the Q_{10} of

increase in wall plasticity, as this Q_{10} may be "blended"° with that of wall stretching which is presumably close to one. It is therefore quite possible that the Q_{10} of actual action is as high as that of respiration, although a direct measurement is impossible.

This indication that growth substance action is a metabolic process continuous during wall extension makes more understandable the result of Part IV., i.e. that the "efficiency" of growth substance possesses a Q_{10} of 2.2. That this temperature coefficient is almost identical with that of respiration further suggests that the wall extension due to one growth substance molecule depends directly upon the magnitude of a respiratory process.

Concerning the process of action a few in-vitro experiments have been made. The effects of growth substance upon the dehydrogenase system of coleoptiles was extensively investigated using the usual Thunberg technique. A slight increase in the reduction time of methylene blue in the presence of growth substance was found, but

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°Footnote: It may readily be seen how two coupled processes of different temperature coefficients may yield an intermediate one. For example consider the reactions $A \xrightarrow[a]{} B \xrightarrow[b]{} C$. The temperature coefficient of a is 2, that of b is 1. For a, $\frac{dB}{dt} = K_2A - K_3B$ at T_1 and $\frac{dB}{dt} = \frac{K_2A}{2} - K_3B$ at T_2. For b $\frac{dC}{dt} = K_3B$ at T_1 and $\frac{dC}{dt} = K_3B$ at T_2. In comparison with its rate of production, B is used more rapidly at T_2 and therefore the concentration of B at the steady state will be less. Therefore the final rate of production of C will be less at T_2, and, depending upon the relative values of K_2 and K_3 the apparent coefficient of the entire process will assume values intermediate between 1 and 2 for a longer or shorter time after a change from T_1 to T_2.

this effect could be duplicated with other unsaturated acids such as crotonic and ricinoleic which have no growth substance-like activity upon cell extension. It was conceivable that growth substance could be the heat stable, dializable, coenzyme of carboxylase (Wetzel, 1932) but an extended series of manometric experiments showed that this is not the case. Growth substance also apparently cannot replace the catechol component of the coleoptile oxidase system. This type of in-vitro experiment, seems nevertheless, a possible fruitful method of future attack.

6. Discussion

The significant results of this part may be summarized as follows:

1) Over the grand period of the coleoptile, total respiration and total elongation run qualitatively parallel. Over the length of a given coleoptile, respiration per unit length is almost constant, altho elongation per unit length varies. Neither over the grand period or in a given coleoptile does respiration per unit protein or per unit dry weight parallel elongation.

2) Cyanide decreases growth and respiration to about the same extent. Lower temperature decreases respiration more than elongation. Neutral buffers decrease elongation without exhibiting any effect upon the magnitude of respiration.

From 1 and 2 it is necessary to conclude that decreases in respiration decrease elongation but not the reverse, i.e. no considerable portion of the respiration is a result of elongation processes.

The dependance of growth upon the existence of a large respiration may be viewed in two ways

a) the entire respiration is a "formal prerequisite of growth" (Pfeffer);

b) one of the processes of growth is itself a respiratory process but one of small oxygen uptake.

It has been shown in the introduction that the entire respiration cannot be needed for the supply of energy needed in growth. a) can give therefore no clear idea of why a reduction of respiration by HCN for example should decrease elongation to even approximately the same extent. This brings up the question as to in what way respiration might be a "formal prerequisite" for growth. It is clear that it can be necessary only if it performs functions which are essential to growth. The principal function it performs is the production of heat, which it is difficult to visualize as useful for elongation. It is likely therefore that only a portion of the respiration is needed for actual growth, and a) would become identical with b).

Experimentally, the distinction between a) and b) is also difficult if not impossible. Upon a), growth is cut when respiration is cut because the whole respiration is essential. Upon b), elongation is cut if respiration is cut because the two kinds of respiration have similar properties. Upon a) elongation can cease and respiration continue because the latter is a prerequisite of the former. Upon b) elongation can cease and respiration continue because either of somewhat different properties of the two respirations or because of the inhibition of other portions of the elongation process.

Concept a) cannot then be either theoretically or experimentally strictly differentiated from concept b). It would seem quite desirable then to avoid the ambiguity of meaning in statement

a), and to express the necessity of respiration for growth in the statement that a process of respiratory nature is one of the component processes of elongation.

One of the most signfficant results of the present part is the demonstration that growth substance action probably takes place continuously during wall extension. This continuous action cannot be due to intussusception of new material (Part V). The remaining possibilities are a) that it affects orientation of wall micelles b) that it causes production of substances havingconsiderable influence upon the colloidal nature of the cell wall. The first possibility may be studied by X-ray methods. The second possibility may be studied only by investigation of the effects of growth substance upon metabolism and the relation of this to the cell wall. The present part has succeeded only in outlining the general nature of the metabolic processes which should be studied.

Summary

The results of the present part may be summarized as follows:

1.Over the grand period of the coleoptile,respiration parallels qualitatively rate of elongation.Respiration per unit dry weight,per unit protein,or per unit length exhibits no clear correlation with growth rate.

2.Respiration falls steadily from tip to base of the coleoptile,in contrast to the growth rate which passes through a maximum.Over the length of the coleoptile,then the two processes exhibit no clear correlation.

3.Concerning the relation of growth and respiration in the excised rapidly growing zone,the following facts have been established;

a.The growth of the section falls to about 2 per cent of its initial value,under the experimental conditions,while the respiration falls only about 17 per cent.

b.Inhibition of respiration by HCN is accompanied by a corresponding inhibition of growth.

c.Decrease of temperature,however,causes a larger per centage decrease in respiration than in growth.

d.Changes in growth rate due to changes in the growth substance supply are not accompanied by any detectable change in the rate of respiration

From a.,b.,c.,and d.,it is clear that decreases in respiration are always accompanied by decreases in growth rate.It is suggested that this due to the fact that one or more of the processes of growth are of a respiratory nature,although processes of small oxygen uptake.

e.Growth substance has no detectable effect upon the respiratory quotient,although changes of the pH of the buffer in which the coleoptiles are suspended cause large variations of this quantity.

f.The growth of sections under the present conditions is limited by the action of growth substance after its entry into the section and not by the rate of this entry.It seems probable that the action of growth substance is an aerobic process which must go on during the actual plastic deformation of the cell wall.

TABLE 45.

EFFECT OF CUTTING UPON THE RATE OF RESPIRATION OF COLEOPTILE SECTIONS°

Experiment	No. cut surfaces per section	Q_{O_2} per section	No. cut surfaces per section	Q_{O_2} per section
1	2	3.08	4	3.06
2	2	2.94	8	2.80

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

TABLE 46.

COMPARABILITY OF DUPLICATE SAMPLES OF COLEOPTILE SECTIONS

Expt.	Vessel	Q_{O_2} per section. 1	2	3	4	mean	Mean variation	Mean variation as % of mean
1		1.40	1.43	1.42	1.30	1.40	0.037	2.7
2		1.68	1.72	1.56	1.64	1.65	0.050	3.0
3		1.35	1.32	1.29	1.33	1.32	0.018	1.3

TABLE 47.

SUMMARIZED DATA OF GRAND PERIODS OF GROWTH AND RESPIRATION

Age hours	Height mm	CO_2 per plant	Dry wght. per plant mgs.	Protein per plant mgs.
12 (embryo)	2.35	0.50	0.254	0.08
24 (embryo)	2.80	1.49	0.304	0.12
36 (embryo)	3.95	2.51	0.475	0.18
36 (shoot)	1.34	——	0.161	——
48 (embryo)	13.80	7.18	1.000	0.38
48 (shoot)	2.82	1.34	0.257	0.12
60 (shoot)	6.6	2.95	0.70	0.24
72 (shoot)	18.7	6.82	1.81	0.54
72 (coleoptile)	16.0	5.62	0.915	0.25
84 (coleoptile)	27.3	6.79	1.32	0.335
96 (coleoptile)	46.2	9.25	2.22	0.49
108 (coleoptile)	46.0	9.19	2.42	0.51
120 (coleoptile)	46.4	8.05	2.49	0.49

TABLE 48

SUMMARIZED DATA FOR GRAND PERIODS OF COLEOPTILE ALONE

Age hrs.	Height mm.	Q_{O_2} per plant	Dry wt. mgs.	Protein mgs.	% protein	12 growth mm.	% growth	Q_{O_2} per mg. dry wt.	Q_{O_2} per mg. prot.	Q_{O_2} per mm.
12	0.64	0.14	0.04	—	—	—		3.5	—	0.22
24	0.78	0.42	0.05	—	—	0.14	19.7	8.4	—	0.54
36	1.09	0.70	0.08	—	—	0.31	33.3	8.7	—	0.64
48	2.33	1.10	0.12	0.055	46	1.24	72.5	8.4	20.0	0.47
60	5.5	2.42	0.35	0.11	32	3.2	82.0	6.9	22.0	0.44
72	16.0	5.62	0.915	0.25	27	10.5	97.8	6.1	22.4	0.35
84	27.3	6.79	1.32	0.33	25	11.3	52.4	5.2	20.5	0.25
96	46.2	9.75	2.22	0.49	22	18.9	51.4	4.4	19.9	0.21
108	46.0	9.19	2.42	0.51	21	-0.2	0	3.8	18.0	0.20
120	46.4	8.05	2.49	0.49	20	0.4	0	3.2	16.4	0.17

TABLE 49

DISTRIBUTION OF RESPIRATION IN THE COLEOPTILE

Experiment	Zone	5 mm. tip Q_{O_2}/mm.	Next 6 mm. Q_{O_2}/mm.	Next 6 mm. Q_{O_2}/mm.	Base Q_{O_2}/mm.	Av. plant height mm.
1		0.26	0.24	0.22	0.25	29
2		0.27	0.26	0.26	0.22	31
3		0.27	0.31	0.30	0.30	26
Mean		0.27	0.27	0.26	0.26	
Mean Q_{O_2} in 1% fructose		0.30	0.29	0.29	0.25	

TABLE 50.

DISTRIBUTION OF PROTEIN AND DRY WEIGHT IN THE COLEOPTILE

Experiment	Zone	5 mm. tip	Next 6 mm.	Next 6 mm.	Base
		mgs. protein per mm.			
1		0.96×10^{-2}	1.21×10^{-2}	1.30×10^{-2}	1.39×10^{-2}
2		1.03	—	1.43	1.39
3		1.28	1.16	1.23	1.53
4		1.13	1.27	1.42	1.63
Mean		1.10×10^{-2}	1.21×10^{-2}	1.36×10^{-2}	1.47×10^{-2}
		dry weight mgs. per mm.			
1		3.9×10^{-2}	4.8×10^{-2}	5.4×10^{-2}	5.5×10^{-2}
2		4.0×10^{-2}	4.8×10^{-2}	5.2×10^{-2}	5.6×10^{-2}
3		3.7×10^{-2}	5.1×10^{-2}	5.2×10^{-2}	5.5×10^{-2}
Mean		3.9×10^{-2}	4.9×10^{-2}	5.3×10^{-2}	5.5×10^{-2}
Mean % protein		28	25	25.5	27

TABLE 51

INHIBITION OF RESPIRATION BY HCN

Expt.	HCN conc. molal	% Inhibition $5x10^{-5}$	$1x10^{-4}$	$2x10^{-4}$	$4x10^{-4}$	$8x10^{-4}$
1		22	28	60	79	100
2		7	51	63	76	80
3		22	43	60	75	84
4		28	35	68	89	90
5		23	61	76	83	90
6		12	71	84	80	88
Mean		19±3	45±5	67±4	81±2	89±3

TABLE 52.

INHIBITION OF ELONGATION BY HCN

Expt.	HCN conc. molal	% Inhibition $5x10^{-5}$	$1x10^{-4}$	$2x10^{-4}$	$4x10^{-4}$	$8x10^{-4}$
1		30	51	67	71	87
2		34	42	57	67	81
3		30	38	58	69	—
4		31	—	—	69	—
Mean		31	44	61	69	84
Total plants		30	18	18	30	12

TABLE 53.

AVERAGE GROWTH RATES IN VARIOUS BUFFER SOLUTIONS

Solution	% Elongation during hour		
	1	2	3
pH 4.1	5.9	3.5	2.3
pH 4.1 + g.s.	7.4	6.9	4.1
pH 7.2	2.1	0.6	0.5
pH 7.2 + g.s.	2.6	2.2	2.1

TABLE 54.

EFFECT OF pH OF EXTERNAL SOLUTION UPON RESPIRATION

Expt.	pH 4.1 Q_{O_2}/section	pH 7.2 Q_{O_2}/section
1	1.89	1.76
2	1.38	1.47
3	1.60	1.49
4	1.45	1.57
5	1.68	1.67
6	1.62	1.46
7	1.39	1.38
8	1.46	1.28
9	1.49	1.57
10	1.48	1.36
11	1.37	1.31
Mean	1.53±.05	1.49±.05
Mean variation as % of mean.	8	8

TABLE 55

EFFECTS OF TWO DIFFERENT GROWTH SUBSTANCE PREPARATIONS UPON RESPIRATION

Experiment	Without growth substance QO_2		With growth substance QO_2	
	1 hr.	2 hr.	1 hr.	2 hr.
	Activity $2x10^{-6}$ mgs./unit			
1	1.05	1.12	1.13	1.33
2	1.68	1.60	1.78	2.08
3	1.52	1.43	2.05	1.81
	Activity $3x10^{-7}$ mgs./unit			
1	1.65	1.49	1.74	1.48
2	1.52	1.43	1.53	1.38
3	1.47	1.24	1.37	1.17

TABLE 56.

EFFECTS OF ALANINE AND HISTIDINE ON RESPIRATION

Time hr.		Rate of oxygen uptake in %			
1		100	100	100	100
	Solution added	nothing	1% fructose	0.05% alanine	0.05% Histidine
1		95	107	94	77
2		94	98	91	75
3		86	92	87	75

TABLE 57

EFFECTS OF LOW TEMPERATURE ON GROWTH AND UPON RESPIRATION

Length of Period, hrs.	Expt.	25°		10°		$\frac{\text{Total } O_2, 25°}{\text{Total } O_2, 10°}$	$\frac{\text{Total growth, } 25°}{\text{Total growth, } 10°}$
		Total O_2 mm^3/section	Growth %	Total O_2 mm^3/section	Growth %		
2	1	2.24	13	0.63	5	3.6	2.6
	2	1.95	14	0.53	7	3.7	2.0
	3	2.10	16	0.64	9	3.3	1.8
	4	2.38	15	0.60	8	4.0	1.9
Mean		2.18	14.5	0.60	7.2	3.55	2.1
4	3	3.88	22	1.25	11	3.1	2.0
	4	3.84	19	1.37	14	2.8	1.4
Mean		3.86	20.5		12.5	2.95	1.7
10	1	9.00	21	3.23	14	2.8	1.5
	2	——	22	——	17	——	1.3
Mean		9.00	21.5	3.23	15.5		1.4

TABLE 58.

SUMMARY OF RESPIRATION RATES AT 10° and at 25°

Experiment	25° O_2/section/2hrs.	10° O_2/section/2hrs.
1	2.04	0.56
2	2.35	0.58
3	2.36	0.64
4	2.09	0.57
5	1.98	0.62
6	1.92	0.62
7	1.80	0.63
8	2.24	0.50
9	2.13	0.66
Mean	2.10	0.60
Respiration	$Q_{15} = 3.5 \pm 0.1$	$Q_{10} = 2.6$
Elongation (7 detns.)	$Q_{15} = 2.1 \pm 0.1$	$Q_{10} = 1.6$

TABLE 59.

EFFECT OF HCN UPON RESPIRATION OF SECTIONS FROM YOUNG AND FROM OLD COLEOPTILES

Expt.	HCN conc. molal	84 hour coleoptile					108 hour coleoptile				
		0	1×10^{-4}		4×10^{-4}		0	1×10^{-4}		4×10^{-4}	
		Q_{O_2}	Q_{O_2}	% inhib.	Q_{O_2}	% inhib.	Q_{O_2}	Q_{O_2}	% inhib.	Q_{O_2}	% inhib.
1		1.56	0.66	58	0.37	76	0.92	0.74	20	0.63	69
2		1.42	0.85	40	0.34	76	1.04	0.83	21	0.65	63

TABLE 60

INHIBITION OF RESPIRATION BY HCN IN SOLUTIONS OF pH 4.1 and pH 7.2

Experiment	HCN conc. molal	% inhibition pH 4.1		pH 7.2	
		$1x10^{-4}$	$4x10^{-4}$	$1x10^{-4}$	$4x10^{-4}$
1		31	78	16	55
2		47	76	30	58
3		76	85	20	58
4		76	82	20	57
5		65	89	30	62
Means		59 ± 8	82 ± 2	23 ± 4	58 ± 1

TABLE 61

HCN SENSITIVITY OF ELONGATION AT pH 4 and pH 7.

pH	HCN conc. molal	% Inhibition $1x10^{-4}$	$4x10^{-4}$	Total plants
4	(Table 52)	44	69	—
7		50	71	24

TABLE 62

RESPIRATORY QUOTIENTS IN SOLUTIONS
OF pH 4.1 and 7.2

pH 4.1		pH 7.2	
Expt.	R.Q.	Expt.	R.Q.
1	1.05	1	0.76
2	1.07	2	0.99
3	1.09	3	0.97
4	1.08	4	0.62
5	1.04	5	0.74
6	1.04	6	0.77
7	1.16	7	0.85
8	1.05		
9	1.07		
10	1.06		
11	1.05		
Mean	1.07 ± .01		0.81 ± .06

TABLE 63.

EFFECT OF GROWTH SUBSTANCE UPON THE RESPIRATORY QUOTIENT

(30 standard sections)

Expt.	Solution	Preformed CO_2	Net gas exchange during Expt.	Net gas exchange on adding acid	Total gas exchange	O_2 uptake	R.Q.	Growth
		mm^3	mm^3	mm^3	mm^3	mm^3		%
1	pH 4.1	0.0	+39.0	+12.3	+51.3	175.0	1.29	17.7
	pH 4.1 + g.s.	0.0	+38.2	+13.0	+51.2		1.29	20.7
2	pH 7.2	0.0	-62.5	+21.4	-41.1	243.0	0.83	6.0
	pH 7.2 + g.s.	0.0	-70.9	+27.4	-43.5		0.82	17.3
3°	distilled H_2O	+2.6	- 9.4	+ 1.9	- 9.1	228.0	0.96	8.0
	distilled H_2O + g.s.	+2.6	-6.8	+1.3	- 8.1		0.96	21.0

° 1 % fructose in both solutions.

TABLE 64

EFFECT OF TEMPERATURE UPON GROWTH SUBSTANCE ENTRY

Pretreatment	Plants	Elongation at 25° in H_2O % in 2 hours		Q_{10}
2 hrs. with 1000 u/cc blocks				
at 10°	20	from 10°	7.5	1.0
at 25°	20	from 25°	6.9	

Pretreatment	Plants	Bending at 25°, 1 hr. 250 mg. riders	Elastic bending	Plastic bending	Q_{10}
2 hrs. with 1000 u/cc solution					
at 10°	24	from 10°	4.3	11.0	1.0
at 25°	24	from 25°	3.2	11.2	

TABLE 65

EFFECT OF pH UPON ELONGATION AFTER ENTRY OF GROWTH SUBSTANCE

Pretreatment		Elongation in % at 25° C. 2 hr.	4 hr.	8 hr.
2 hr. with 1000 u/cc blocks at 25°	pH 4.1	9.4	4.6	3.0
	pH 7.2	4.7	1.4	1.2

TABLE 66

EFFECT OF TEMPERATURE UPON ELONGATION AFTER GROWTH SUBSTANCE ENTRY AT 25°

Pretreatment	Plants	Elongation in % per 2 hr.		Q_{10}
2 hr. with 1000 u/cc blocks at 25°	10	at 10°	2.8	1.4
	10	at 25°	4.3	

Conclusions

Detailed summaries have been given at the ends of each of the sections and it will neccessary to outline only the general importance of the present work for the knowledge of cell elongation.

1. The growth substance controlling cell elongation is a true phyto-hormone and not a structural component of the cell wall.
2. It is impossible that the elongation of the Avena coleoptile is by the intussusception of new material. It is due then, to plastic stretching.
3. The action of growth substance is not as has been suggested directly upon the properties of the cell wall. It is indirect and through the functions of the protoplasm.
4. The action of acids upon elongation is due to the depression of the ionization of the weak acid, growth substance, which is principaly active in causing cell elongation when in the non-dissociated form. Growth substance itself has no effect upon the acid metabolism of the coleoptile. Effects of acid upon hydration and imbibition pressure of the protoplasm, long disscussed in the literature, have in the present case only minor importance.
5. Both transport and action of growth substance depend upon respiration-like processes, and this is probably the reason for the well known dependance of the growth of aerobic plants upon the presence of respiration. The existance of the entire aerobic metabolism for the supply of growth energy is unnececessary.

6. The action of growth substance in increasing the plasticity of the cell wall takes place continuously during wall extension.

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