THE MECHANISM OF THE ACTION OF INDOLE-3-ACETIC ACID ON THE WATER ABSORPTION BY AVENA COLEOPTILE SECTIONS

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CONTENTS

GENERAL INTRODUCTION

The mechanism of the growth by cell extension has been a debatable point for many years, in fact since the early days of plantphysiology. The final visible outcome of cell extension is a permanent increase in cell volume, accompanied by a permanent increase in wall area. This seems to be a rather simple phenomenon. Further investigation, however, revealed the difficulty of establishing the exact sequence of events leading to these final results. The initiation of elongation was attributed to a host of different factors, for instance increase in wall elasticity (SACHS), active intussusception of wall substances (PFEFFER) etc. An extensive survey of this older literature is given bij HEYN (1940).

After the discovery of the growth hormone by WENT (1928) and its subsequent isolation by KÖGL and HAAGEN SMIT (1931) the situation became even more complicated. The problem of the mechanism of the action of growth substances demanded a solution. The fact that in most experiments cell extension takes place almost exclusively by the absorption of water, was a lead for attempts in this field of research. Water enters a cell only as a response to a positive suction force. Therefore a positive suction force must be maintained during all stages of elongation. Growth substances may stimulate elongation by disturbing the dynamic equilibrium which exists in the cell. This effect can be attained along two different ways: an increase in the direct forces attracting water into the cell, for instance an increase in the osmotic value of the cell sap or an increase in the imbibitional forces of the cell colloids, or a decrease in the countering forces, for instance a lessening of the wall pressure, which is an inwardly directed hydrostatic pressure originating as a reaction of the elastically stretched cell wall.

The greater part of research has been centered about the latter possibility, which means a change of the properties of the cell wall. Important work has been done by HEYN (1931–1934, 1940). He found that the growth rate is connected with the plasticity of the cell wall. Growth was supposed to be mainly dependent on this property, which was regulated by growth substances. SÖDING (1931–1934, 1952) did not find such clear-cut correlations. He also maintained the im-

portance of the plasticity, but expressed as his opinion that the role of plasticity had been exaggerated.

The majority of the authors reject a change in elasticity as an initiator of growth. Some, however, are of the opinion that it may be important in the first phase of elongation: BURSTRÖM (1942, 1951).

Concerning the first mentioned possibility it can be said that only a few determinations of the suction force are available. The determinations of the osmotic value show that it is not increased through growth substances. A distinction must be made between the values found for intact seedlings and those determined on isolated tissues. In the latter the decrease in the osmotic value is more evident than in the former.

In later years the theories employing the action of growth substances on the cell wall appeared to give too simple a conception of the real facts. It was impossible to explain the optimum curve and the various actions of growth substances (correlative inhibition, root formation, cambium activation) with an effect on the cell wall alone. Moreover, FREY-WYSSLING (1945) and WIRTH (1946) found that a synthesis of protoplasm components and wall substances accompanies the elongation. Therefore protoplasmic activity joins in, and at least part of the action of growth substances should be localized in the protoplasm, which is supported by investigations on the influence of indole-acetic acid on the metabolic activity of living cells. A stimulating effect on respiration was demonstrated by KELLY (1947), ANKER (1951, 1953) and other investigators.

One important property of the living cell, the permeability, has not yet been touched upon. KONINGSBERGER (1942, 1947) and VELDSTRA (1944) introduced the hypothesis that growth substances act on the ectoplast and change the permeability to water. This is further supported by the relation between structure and action of growth substances. (VELDSTRA, 1944). Although VELDSTRA and BOOY (1949) and BOOY and VELDSTRA (1949) found that the theory is not quite satisfactory, the idea has not yet been dismissed that growth substances influence, as their primary effect, the protoplasmic properties through a physico-chemical action on its colloidal constituents. Recently ANKER (1953) suggested that growth substances affect inner lipophilic protoplasmic films.

In the present investigation the problem of the mechanism of the action of growth substances is approached by considering the existing possibilities for a change in the rate of water intake. It seemed worth while to carry out determinations of these factors on the same material, as which were chosen for *Avena* coleoptile sections. After establishing the experimental conditions the permeability to water was investigated. No increase in the permeability to water, which might facilitate the water absorption, was found.

Next an investigation was made into the relation between the presence of growth substances and the magnitude of the suction force, in combination with determinations of the osmotic value. These experiments showed that the osmotic value decreases in proportion to

the increase in length of the sections. Additionally, however, it appeared in these experiments that the wall properties must be changed after treatment with indole-3-acetic acid. A detailed study of this effect was made and the effect of substances, which influence metabolism, on the action of indole-acetic acid was examined. The results show, that a link exists between the metabolic activity and the change in wall properties. This means that the wall is changed by growth substances through the intermediary of the protoplasm. It is held that the synthesis of new wall materials is affected by growth substances. In view of other data available in the relevant literature it seems most likely that the action of growth substances is primarily a physico-chemical one. This is supported by the permeability experiments reported in this paper. The existence of an active absorption of water, as suggested by some authors, e.g. THIMANN (1951), is rejected because the available evidence is considered insufficient. Of course water uptake caused through an active growth of the cell wall is "active", as it takes energy, but it differs from the generally accepted concept of "active water absorption".

MATERIAL and GENERAL METHODS

The experiments were carried out with sections excised from the coleoptiles of four-day-old etiolated seedlings of Avena sativa, variety "Victory" (Sveriges Utsädeförening, Svalöf, Sweden). The unhusked seeds were soaked in tap water for one hour and grown in moist sawdust in complete darkness at 23° C and 96 % relative humidity. All manipulations were carried out in the same darkroom in orange light (Schott OG2 filter), which is phototropically inactive.

Generally the used coleoptiles had a length of about 2 cm. They were placed in a coleoptile microtome after VAN DER WEY (1932) and 4-mm tips were cut and rejected. The next 2½-mm sections (4–6½ mm from the tip) were employed in the experiments. The primary leaf was removed before or after cutting the sections. In the latter case the primary leaf was pushed out of the sections with a thin glass rod, while in the former case the procedure described by VAN DER WEY (1932) was used: part of the base of the coleoptile was removed with a pair of decapitation scissors, after which the primary leaf could be pulled out. Unless otherwise stated these 2½-mm sections without primary leaf were used.

The permeability determinations were carried out with the electromagnetic diver method described by BUFFEL (1952). A detailed description is given in chapter 2. The heavy water (D₂O) used in these experiments was supplied by the Norsk Hydro-elektrisk Kvaelstof-aktieselskab, Oslo, Norway.

In the greater part of the experiments the section test after BONNER (1933) was employed, as modified by VAN SANTEN (1940) and RIETSEMA (1949, 1950). Thus the sections were placed on a slide by means of a narrow vaseline strip and the solutions were aerated during the test. This technique was adapted, when necessary, to the

needs of this investigation. Generally the test was performed without the use of buffer mixtures to keep circumstances as simple as possible. More detailed methodical data can be found in the different chapters.

Two different indole-3-acetic acid (I.A.A.) preparations were available, one from Schering-Kahlbaum (Berlin) and the other one from the Amsterdamse Kinine Fabriek (Amsterdam). The indole-acetic acid was solved by means of gentle heating.

The water used in the experiments was distilled over glass.

CHAPTER I

WATER ABSORPTION

1. LITERATURE

The work on the influence of I.A.A. on the intake of water by tissues has been mainly restricted to storage tissues. Only a few investigators included actively growing tissues in their experiments. Conclusions valid for these storage tissues do not necessarily hold for growing tissues, although it seems that they do. This should be well borne in mind.

This phenomenon has been discovered by REINDERS (1938, 1942). She found that water absorption by potato tuber discs is an aerobic process, promoted by I.A.A. which also causes an increased loss in dry weight. The influence of I.A.A. was found in distilled water. REINDERS proposed as probable causes a stimulation of the respiration, which might bring about an increased osmotic value, or a possible greater extensibility of the wall.

Her results were confirmed by other authors and the experiments were continued with a view to elucidating the mechanism of action of I.A.A. COMMONER and MAZIA (1942) thought about an increase in the osmotic value of the cell sap. This could be brought about through anatonosis or endosmosis. They stressed the latter possibility and tried to find a correlation between salt uptake and water uptake. Both processes were promoted by I.A.A. to the same degree. The authors obtained these results both with potato tuber discs and Avena coleoptile sections. A further analysis by COMMONER, FOGEL and MULLER (1943) in combination with the results of other investigators led to the hypothesis, that I.A.A. stimulates the respiration. This would produce an increased salt uptake which in its turn is compensated by an increased water uptake. The final result will be an increase in cell volume. COMMONER and MAZIA (1944) gave further data supporting this supposition for Avena coleoptiles and for potato tuber discs. They found an influence of C_4-acids in the presence of I.A.A. on salt and water uptake.

This conception, however, seems to be too simple. The exact time sequence for salt and water uptake was not determined and compared. There is no proof that the hypothesis is quantitatively adequate and it does not explain the promoting effect of I.A.A. in distilled water as found by REINDERS (1942) and VAN OVERBEEK (1944). VAN

OVERBEEK criticizes the work of COMMONER et al. because they performed experiments with sucrose for 4 days without maintaining aseptic conditions. VAN OVERBEEK determined the osmotic value, which appeared to decrease slightly during the experiment, both with growth substance and without. The freezing point depression is lower in the discs treated with growth substance than in the controls, but the differences are rather small. These determinations were naturally carried out with press sap of tissues and are, therefore, not quite satisfactory. This author eliminates the role of starch hydrolysis with the argument that the osmotic value does not increase. This is premature for he did not carry out determinations of the amount of osmotic active material in the cell. Even then there remains the possibility that the greater part of the lower carbohydrates originating from starch hydrolysis has been consumed in respiration. VAN OVERBEEK considers a decrease in wall pressure or an increase in "the anomalous components of the suction force" as most important, the latter being the most probable cause.

LEVITT (1948) gives a thermodynamical consideration of the phenomenon and a series of experiments.

From the results of his experiments he deduces that an active absorption can be eliminated. LEVITT found water uptake at low temperatures (0°–2° C) and with KCN, and not a loss of water as he had expected in the case of active absorption alone. This author supposes that I.A.A. increases the plasticity of the cell wall. He confirms VAN OVERBEEK's results in also finding lower freezing point depressions in discs treated with I.A.A.

All experiments show that the enhanced water uptake is connected with respiration. Without oxygen there is no water uptake. This is clearly demonstrated by KELLY (1947), who determined water uptake and respiration in the oat coleoptile. Azide, cyanide and iodoacetate inhibited both processes to the same degree. This is at variance with the results of LEVITT (1948). The stimulating action of I.A.A. was prevented by azide and iodoacetate.

HACKETT and THIMANN (1950, 1952) also studied the effect of various inhibitors: azide, arsenite, dinitrophenol and fluoroacetate. All these substances inhibit the water uptake. I.A.A. and naphthalene acetic acid promote the process, the latter being more effective. Reduction of the oxygen pressure also reduces the uptake of water. The authors conclude that the water uptake of potato tissue both in the presence and in the absence of externally added growth substance is linked to the general aerobic metabolism and corresponds closely to elongation in etiolated seedlings.

THIMANN (1951) suggested that the enhanced water uptake might be caused through a greater plasticity of the cell wall, brought about by an oxidative process, or by an active pumping of water into the cell. He prefers the latter possibility, considering that, after growth inhibition through immersion of the tissues in a solution with a fairly high osmotic value containing a growth substance, the growth rate does not increase suddenly when this solution is replaced by distilled

water also containing growth substance. This conclusion is not justified if the wall meanwhile has been changed because new material has been formed and interwoven. In that case secondary layers may have been laid down, solidifying the new parts of the primary wall. The same process will take place in cells inhibited by an external suction force and when this force is removed no sudden increase in growth rate can be expected. THIMANN's conclusion would be correct if the change in wall properties is brought about through a lasting loosening of the micellar structure.

HACKETT and THIMANN (1953) studied the relation between respiration and water absorption. They used dinitrophenol as an inhibitor and they concluded that water uptake is dependent on a sufficient supply of high-energy phosphate bonds. This conclusion is supported by BONNER and BANDURSKI (1952). HACKETT and THIMANN find a relation between water uptake and respiration, but not between the rate of water absorption and the rate of respiration. The relation between both processes is not yet clear.

HACKETT (1952) determined the osmotic changes during water uptake of potato tissue cryoscopically. The effect of naphthalene acetic acid on the fresh weight and on the shift of the freezing point depression appeared to match.

This means that the osmotic value of the cell sap decreases in direct proportion to the increase in fresh weight. The results of VAN OVERBEEK (1944) and LEVITT (1948) are thus confirmed.

A close relation between water intake and oxybiosis was also established by BRAUNER, BRAUNER and HASMAN (1940) and by BRAUNER and BRAUNER (1943).

HSIANG (1951) studied the water uptake by discs cut from the floral parts of orchids. She found an influence of growth substances and a dependency of water absorption on an aerobic process.

AUDUS (1952) criticizes the results of many other investigators. He stresses that it is necessary for an exact interpretation of experiments about the action of inhibitors to compare growth rates and not total growth determinations. This is the more important when the influence of inhibitors on two different processes, for instance respiration and growth, is compared in order to establish a relation between these two processes. In this respect the results of many authors must be reconsidered.

Frequently the influence of different substances on water uptake, or growth, and their effect on respiration are not determined after the same time of treatment. A comparison of the values found will give an erroneous impression of the relation between both processes. AUDUS shows this for the action of arsenite on growth (water uptake) and respiration in pea stem sections.

The evidence surrounding the effect of growth substances on the water intake of tissues is thus very conflicting. The connection of the absorption of water with the respiration is evident, but the exact relation between the two processes is uncertain. There is as yet insufficient direct proof that oxygen and respiration affect an active

water absorption. It is possible that the metabolic activity changes the wall properties and that the greater water uptake can be accounted for osmotically. Some authors point out that the permeability to water can be affected: KONINGSBERGER (1942, 1947), VELDSTRA (1944), VON GUTTENBERG and KRÖPELIN (1947), POHL (1948) and BRAUNER and HASMAN (1949). The greater water permeability however can explain only a difference in rate of uptake and is not able to shift the final equilibrium. Moreover, the permeability to water is already relatively great. Therefore a change in permeability to water cannot be considered as the primary cause of the increase in water absorption.

2. EXPERIMENTS

Water uptake can be measured as the increase in fresh weight of a number of isolated coleoptile sections. A series of experiments was carried out with 2½-mm sections without primary leaf. 30 sections were cut, placed on thin glass rods in 100 ml distilled water in a Petri dish and aerated. The rods with sections were kept under the surface by sticking them to the bottom of the dish with vaseline. After 6 hours the sections were lifted out from the dishes, blotted and weighed and then put in the experimental solutions. After 24 hours the sections were weighed again and the increase the fresh weight was determined. The results shown in table 1 are the average values from 6 experiments.

TABLE 1.
The effect of indole acetic acid on water absorption.

	Total weight mg		Weight per section mg		Increase mg per section	Increase %
	Initial	Final	Initial	Final		
Contr.	62,1	66,6	2,07	2,22	0,15	7,2
1 mg/l	61,5	77,9	2,05	2,60	0,55	26,8
10 mg/l. . . .	63,4	87,8	2,11	2,93	0,82	38,9

The water uptake is enhanced by I.A.A., the highest concentration having the greatest effect. After these preliminary experiments it seemed desirable to determine the I.A.A. concentration that has the maximum effect. Therefore the technique was changed in a manner which allowed more I.A.A. concentrations to be tested in the same experiment.

7½-mm sections (4–11½ mm from the tip) were cut, the primary leaf removed and 30–50 sections floated on the surface of 100 ml distilled water in a Petri dish. After 6 hours the sections were blotted, weighed and placed in the experimental solutions (250 ml). In order to keep the sections under the surface of the solution, they were enclosed in a glass vial (heigth 3½ cm, diameter 4½ cm), the open ends of the tubes being closed with cheese cloth. The solutions were aerated through a Jena G1 glass filter. After 24 hours the increase in fresh weight was determined. Special care was taken to make sure that the solution was removed from the central hole of the sections

TABLE 2

The increase of the fresh weight of sections under influence of I.A.A. in % per section.

I	II	III	0	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	0,5	1	5	10	20	30	40	50	60	100
1†*	6	30	37,7						76,1		78,8		75,4						
2†*	23	32	6,0						4,4		10,2		7,2						
3†	6	30							45,2	49,5	60,6								
4	6	50									48,4	62,1	57,9			57,8	45,2		29,3
5†	6	50									44,5	46,1	50,7			52,1	45,1		11,1
6	6	40	18,6						21,4		37,8		43,7			42,7		45,6	
7	6	40											20,4	21,3	19,3	18,7	19,0	29,8	
8	6	40	18,8		9,5	10,4	9,6	19,8	24,0		41,6					47,6			
9	6	40	10,4										54,5	55,6					
10	6	40	9,4										39,2						
11	6	50	9,5	10,2	9,4	7,8	10,9	14,8											
12§	6	50	10,8		8,0	11,8	10,4												

I.   Number of the experiment.
II.   Time in hours during which the sections remained in distilled water before the experiment.
III.   Number of sections per series.
† Sections with primary leaf.   § 5-mm sections.
* Plants as are grown for the standard Avena test (page 404). The upper row of the table, above the heavy line, shows the concentration of I.A.A. in mg/l.

before weighing. The results of these experiments are collected in table 2.

Table 2 shows that the maximum effect was generally found between 1 and 40 mg/1, with a preference for the higher concentrations. This confirms the results of COMMONER and MAZIA (1942) and agrees more or less to the data of KELLY (1947). The lowest concentration of I.A.A. giving a stimulation of the water uptake is found to be 0,01 mg/1. Further dilutions had no significant effect.

In the greater part of the experiments high concentrations of I.A.A. appeared to be injurious. Fig. 1 shows this very clearly for concentrations higher than 10 mg/1. The sections become twisted and partly flaccid. The sensitivity to I.A.A. differed from one batch of plants
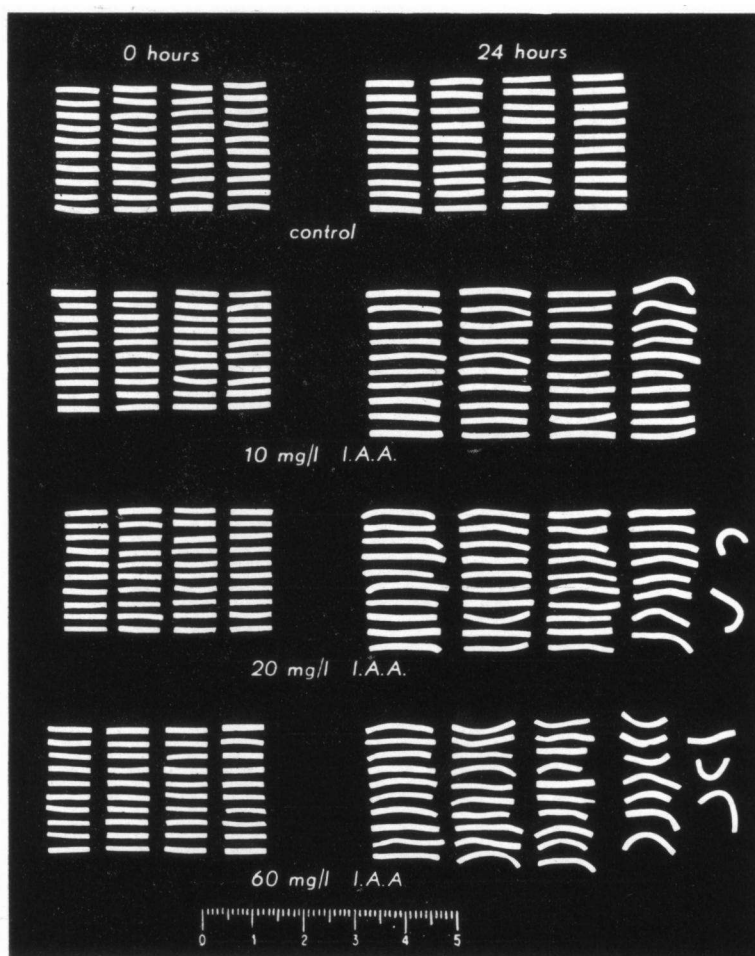

Fig. 1. The injurious effect of high concentrations of I.A.A.

to another. In one experiment (exp. 6 for example) the maximum effect was found at 60 mg/1, while in all the other experiments this concentration was definitely injurious. Therefore in the following experiments 1 and 10 mg/1 will be used almost exclusively.

In some experiments the effect of coumarin was tested, sometimes combined with I.A.A. (Table 3).

TABLE 3
The effect of I.A.A. and coumarin on the procentual increase of fresh weight

	Exp. 1	Exp. 2
Control	4,3	15,2
Coumarin 1000 mg/1.	1,3	6,6
Coumarin · 500 mg/1.		6,6
Coumarin 100 mg/1.	4,1	9,7
I.A.A. 10 mg/1	25,0	
I.A.A. 10 mg/1 plus coumarin 100 mg/1. .	11,0	

The representative experiments in table 3 show that coumarin had an inhibitory influence on the uptake of water and partly suppressed the stimulating action of I.A.A. The same was found when elongation was measured as an indicator for the effect of coumarin (table 4).

TABLE 4
The effect of I.A.A. and coumarin on elongation

I.A.A.	Coumarin	Elongation
—	—	13,3 %
10 mg/1	—	55,8 %
—	100 mg/1	10,4 %
10 mg/1	100 mg/1	18,0 %
—	500 mg/1	12,3 %
10 mg/1	500 mg/1	15,0 %

The concentrations showing inhibition are of the same magnitude as those used by Von Guttenberg and Beythien (1951) in their permeability studies with epidermal cells of *Rhoeo discolor*.

3. Discussion

The experiments described above confirm the facts found by other investigators. I.A.A. strongly promotes the uptake of water, while coumarin has an inhibiting effect. The most favourable conditions for further experiments can be derived from these results. So the most suitable concentration of I.A.A. will be 1 or 10 mg/1. It is also shown that a considerable absorption of water takes place in distilled water. Therefore the experiments can be performed in a very simple medium, no sugars or salts being required. This simplifies the interpretation of the results.

I.A.A. may bring about the increase in water uptake through an increase in the osmotic value or a decrease in the wall pressure as purely osmotic possibilities, or through the stimulation of an active

water absorbing mechanism. A change of the permeability may affect the rate of uptake. The permeability to water will be examined in the next chapter.

<div align="center">CHAPTER II</div>

<div align="center"># THE PERMEABILITY TO WATER</div>

A. INTRODUCTION

From the experiments described in the preceding chapter the conclusion was drawn that I.A.A. enhances the absorption of water by isolated coleoptile sections. In several publications it has of late been suggested that growth substances may affect the permeability to water. Without being the primary cause (page 394) an increase in the permeability may facilitate the water uptake. This can be so only when the permeability functions as a "bottleneck" for the water absorption.

Growth and water uptake of isolated tissues are influenced, for example, by the pH of the medium and by the presence of growth substances. The following survey of the relevant literature discloses that no sufficient data about the effect of pH and growth substances on the permeability of growing tissues are yet available. We will examine, therefore, whether these two factors change the permeability to water. Even if no effect is found, this does not exclude the possibility that growth substances affect lipophilic membranes within the cells, but only the ectoplasm is at the moment accessible to experimental analysis.

B. LITERATURE

1. *pH and the permeability to water*

The influence of pH has been investigated by BRAUNER (1943). BRAUNER found a maximum permeability at an acid pH. He concluded that the optimum pH coincides with the I.E.P. of the protoplasm proteins.

POHL (1948) carried out investigations into the effect of pH on the permeability of *Avena* coleoptile sections. Different pH values were obtained with acetate buffer mixtures after MICHAELIS. These mixtures were diluted 1 : 10. POHL found maximum permeability at pH 4,3. However, the use of acetate buffers must be criticized because it is a well-known fact that acetate is toxic for plant tissues: COGGESHALL (1931), SAKAMURA and KANAMORI (1935), VAN SANTEN (1940), LUNDEGÅRDH (1942). VAN SANTEN proved that even a 1/3000 N acetate buffer damaged the roots of *Helianthus annuus*. The injurious effect increased with increasing acidity. POHL's use of citrate-phosphate mixtures in other experiments does not do away with this objection, for VAN SANTEN (1940) reported that citrate is also toxic.

SEEMANN (1949) studied the effect of pH on the permeability to

water of different adult tissues. He found a single peaked curve, with an optimum at pH 6 or 7. Generally, a plateau region of maximum permeability was found and not a distinct optimum, while the permeability decreased in low and high pH. He could not decide between an optimum corresponding to the I.E.P. of the protoplasmic proteins or an optimum coinciding with neutrality.

VON GUTTENBERG and MEINL (1952) carried out experiments with the epidermal cells of *Rhoeo discolor*. They determined the pH of different I.A.A. solutions and compared the effect of these solutions with the effect of different pH's obtained by adding HCl to distilled water. The greatest permeability was found at pH 5,4. pH 3,7 decreased the permeability but did not kill the cells as did the I.A.A. solutions with a comparable pH (1 gr/1). The experiments were repeated with citric acid over a pH range from 1,3–5,8. In this solution the maximum permeability was found at pH 5,6–5,8. This result does not signify much, because there are no data available about the effect of neutral and alkaline pH. VON GUTTENBERG and MEINL also studied the effect of a di-potassiumcitrate buffer after SÖRENSEN. They found a maximum permeability at pH 5,4–5,6. It seems more in agreement with their data to state that not much difference exists between pH 4,4 and 6,0.

It appears from this survey of the relevant literature that only POHL (1948) carried out permeability determinations with growing tissues and his experiments are not quite convincing. Therefore it seemed worth while to examine the effect of pH on the permeability of growing tissues once more.

2. *Growth substances and the permeability to water*

KONINGSBERGER (1942, 1947) and VELDSTRA (1944) suggested that the primary effect of growth substances might be found in the ectoplasm. They considered the ectoplasm as a complexcoacervate film in the sense of BUNGENBERG DE JONG (1947). Growth substances might act as sensitizers when they are absorbed in the ectoplasm. The result would be a change of permeability.

KONINGSBERGER and co-workers (1947) supported this hypothesis with evidence from studies about the effect of I.A.A. on the rate of deplasmolysis of isolated protoplasts, obtained from the mesophyll cells of the bulb scales of *Allium cepa* (the onion). 0,1 and 1 mg/1 I.A.A. blocked up the permeability to water completely for a few minutes. When the permeability was restored it remained decreased for 1–2 hours. This indicated that I.A.A. affects the protoplasmic membrane.

VELDSTRA (1944) suggested on the ground of his experiments about the relation between structure and activity of growth substances, that growth substances may have a sensitizing effect on coacervates. BOOY and VELDSTRA (1949) and VELDSTRA and BOOY (1949) carried out experiments to test this hypothesis and therefore studied the effect of different growth regulators on coacervates. The type of reaction found met with the expectations, but the quantitative

relations between the opening effect on coacervates and the physiological activity in the pea test were the reverse of those expected. The same was found in the "beet test" employed by VELDSTRA. Therefore the authors were led to consider the possible effect of growth regulators on the ectoplasmic layer as not being the primary cause of the growth reaction. Their experiments show clearly, however, that growth substances are membrane-active.

VON GUTTENBERG and KRÖPELIN (1947) investigated the influence of I.A.A. on the nyctinastic movements of the leaves of *Phaseolus coccineus*. The authors suggested that I.A.A. increases the permeability to water in the swelling tissue of the motile pulvini. Experiments with *Rhoeo discolor* and *Allium cepa* confirmed that the permeability to water is increased under influence of I.A.A.

VON GUTTENBERG and BEYTHIEN (1951) carried out more extensive investigations about the effect of different growth substances, growth inhibitors and some ions on the permeability to water of epidermal cells of *Rhoeo discolor*. When I.A.A. is added during deplasmolysis, the permeability to water is decreased, which is in accordance with the findings of KONINGSBERGER et al. (1947). This inhibition is strongest with 0,1 mg/l I.A.A., but is more persistent at the higher concentrations of I.A.A. The authors stated that plasmolysis is also accelerated through I.A.A.

VON GUTTENBERG and MEINL (1952) tried to analyse the action of I.A.A. They excluded the possibility that the action of I.A.A. is an effect of pH. They suggest an effect of growth substances on the protein components of the protoplasm. But the authors base their conclusions on insufficient and unconvincing experimental data. The results of their swelling experiments do not justify the explanations given.

BRAUNER and HASMAN (1949) studied the water uptake of potato tissue. From a graphical analysis of gravimetric measurements of the suction force they arrived at the conclusion that after short times of exposure to I.A.A. the primary effect of I.A.A. consists in an enhancement of the water permeability, while after longer times of exposure the tensility of the wall has been increased. Against these experiments the objection may be put forward that the graphs used for the analysis have been drawn through three points only.

RUGE (1937) had already found that the permeability is increased after treatment with I.A.A. He found this effect when a considerable amount of elongation had occurred and consequently concluded that the change in permeability is a secondary effect.

The preceding investigations were carried out with adult tissues, which is a disadvantage when growth substances are studied. POHL (1948) made experiments with *Avena* coleoptile sections. He used the osmotic principle for his permeability determinations, dehydrating sections without actually plasmolysing them. POHL interprets different changes of length during a certain time of dehydration after an I.A.A. treatment as differences in permeability. This is not allowed without further consideration, because osmotic conditions or wall properties

may be changed by the action of I.A.A. Experiments to be described in Chapter III and IV demonstrate that this is indeed true, even after 1 hour of treatment with I.A.A.

For the relation between the concentration of I.A.A. and the resulting growth POHL found a double peaked curve during the first hour or so after the addition of I.A.A. POHL explained the first peak found at about 0.08 mg/1 I.A.A. as a result of an increase in the permeability to water. Higher concentrations decreased the permeability to water, but then electro-osmosis would play a part.

Apart from the fact that a change of permeability cannot shift the final equilibrium, objections can be raised against the use of sections immediately after cutting. RIETSEMA (1950) found that a passive water uptake of an osmotic nature plays a prominent part during the first few hours after the immersion of freshly cut sections into a solution, because the sections are not fully turgescent. Conclusions based on measurements during this period must be regarded with extreme caution. In our own experiments (unpublished) we did not find a double peaked curve when using sections which had been immersed in distilled water for 5 hours before the addition of I.A.A.

BUFFEL (1949, 1952) studied the effect of I.A.A. on the permeability to heavy water of isolated *Avena* coleoptile sections. By employing heavy water BUFFEL was able to avoid the use of the osmotic methods (see page 402). He used "aged" sections, this means, sections which have been immersed in distilled water for about 18 hours before they are used in the experiment. The result of these experiments was that I.A.A. has an opening effect of about 20 % in concentrations of 0,1, 1 and 10 mg/1. The increase in permeability is thus very slight and it seems unlikely that this small response can bring about the enormous rise in growth rate. Moreover, it will be desirable to know the response of sections which have not been aged and still show the full growth response.

C. METHODS

1. *Introduction*

The osmotic methods available for the investigation of the permeability to water were rather unsatisfactory. When plasmolysis and deplasmolysis occur injury to the cell is very likely (REINHARDT, 1899. ALBACH, 1930). Some authors avoid plasmolysis, but even then the hydration of the protoplasm and the area or thickness of the membranes will change, which may bring about an altered permeability (HUBER and HÖFLER, 1930. RESÜHR, 1935). Moreover, it is difficult to compare the permeability constant for water with those for other substances because the former is measured as a filtration constant, while the latter are diffusion constants.

The discovery of the heavy hydrogen isotope by UREY, BRICKWEDDE and MURPHY (1932) and its subsequent concentration in the form of heavy water by LEWIS and MACDONALD (1933) offered new possibilities, for D_2O allows a direct determination of the water concen-

tration and consequently of the permeability to water (WARTIOVAARA, 1944). D_2O was introduced in permeability studies by LUCKÉ and HARVEY (1935), followed for instance by KROGH and USSING (1937). WARTIOVAARA (1944) was the first who employed heavy water in permeability experiments with plants (*Tolypellopsis*).

The most serious objection to the use of D_2O is the possibility that the permeability (diffusion) constants for ordinary and for heavy water may be different. Early experiments seemed to support this possibility (BROOKS, 1935. PARPART, 1935.), but later investigations show that this is not correct. Both constants are the same within the experimental variation (WARTIOVAARA, 1944. LØVTROP and PIGOŃ, 1951).

BUFFEL (1949, 1952) developed three different methods for measuring the permeability to heavy water, which methods make use of the changes in reduced weight originating from the exchange of D_2O against H_2O. PIGOŃ and ZEUTHEN (1951) developed a method on the same principle, adapting the Cartesian diver balance after ZEUTHEN (1948) for the purpose.

2. *The electromagnetic diver method*

The method has been described extensively by BUFFEL (1952). Very light glass divers (fig. 2), weighing about 90 mg, their length being about 115 mm, bear a soft iron grain (9) enclosed in the lower end. Below the iron grain the diver (6) is drawn out to a very thin point, which rests on the smooth surface of a glass rod (8) in the narrow part of the diffusion vessel (5). This part of the diffusion vessel passes through the centre part of a solenoid (7). When current passes the coil an electromagnetic field is created, which magnetizes the iron grain of the diver. The iron grain is lifted up and the diver moves upward.

The force excercised upon the diver is, for a given solenoid, proportional to the strength of the current passing through the coil. The force which will be able to lift up the diver must be greater when the reduced weight of the diver is greater. Therefore, the current strength is a measure for the reduced weight.

This principle is worked out in the apparatus in the following way: The solenoid forms part of a circuit which consists of a rheostat and a slide wire resistance for regulating the current strength, an ammeter measuring the current in mA and a telegraph key which allows a rapid making and breaking of the circuit. The current is derived from a battery or from a number of dry cells connected in series.

A number of coleoptile sections without primary leaf and loaded with D_2O is slipped over the narrow upper part of the diver. The diver is put in the diver vessel, which is placed in a water bath at 23° C, its narrow part passing through the coil. The rubber stopper (1) is placed in position and the vessel is slowly filled through a rubber tube (2) from a separatory funnel with a solution not containing any heavy water. The D_2O in the sections is exchanged for ordinary water

and, as a result, the reduced weight of diver plus sections diminishes. By means of the rheostat and the slide wire resistance a current is obtained that creates a magnetic force which is just unable to lift the diver. The ammeter is read and the current strength recorded. The lower part of the diver is observed with a magnifying glass
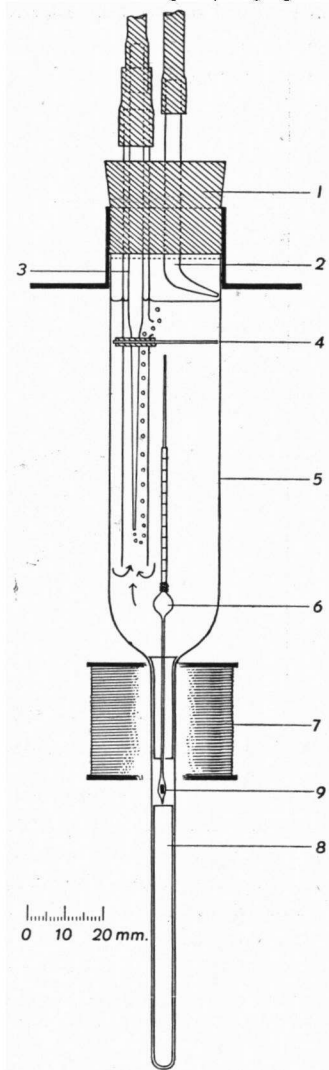
Fig. 2. Diver vessel with diver shown in detail.
1. rubber stopper.
2. filling tube.
3. aerating device.
4. mica plate.
5. diffusion vessel.
6. diver loaded with sections.
7. solenoid.
8. glass rod within the vessel.
9. iron grain.

during the measurements and the aeration is stopped. Measurements can easily be made at one-minute intervals. Care must be taken to measure the initial current strength as quickly as possible. The method is very sensitive and measures reduced weight differences of a few hundredths of mgs.

Each diver – vessel – solenoid combination must be calibrated for reduced weight as a function of current strength. This can be done by loading the divers with small spirals of nichrome resistance wire of known weight and measuring the lifting current. With the help of the calibration curves thus obtained reduced weights can be calculated from the recorded values of the current strength. Fig. 3
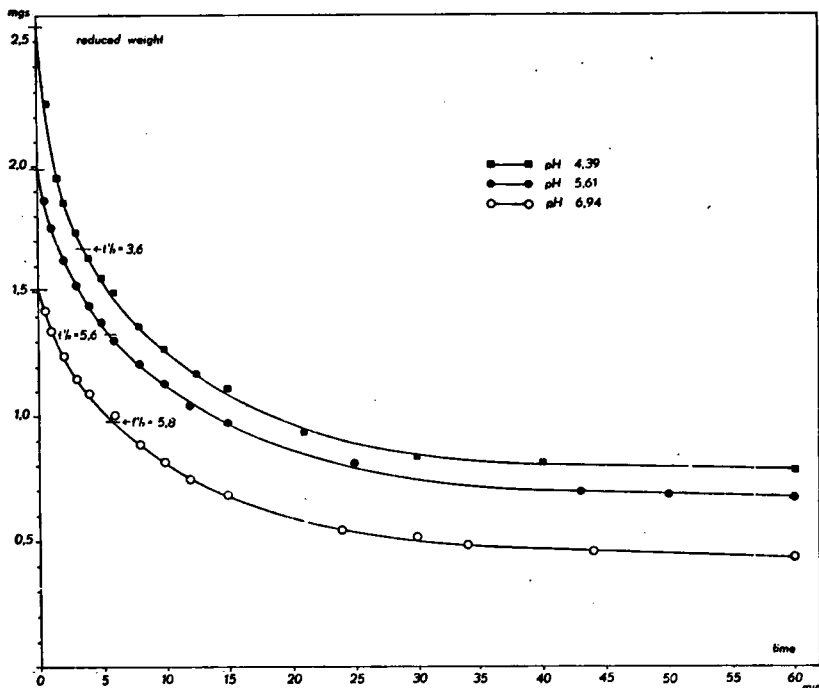
Fig. 3. Diffusion curves obtained with the electromagnetic diver method.

shows diffusion curves obtained in this way with the electromagnetic diver method. For a justified use of the calibration curves it is very important that the distance from the underside of the solenoid to the surface of the glass rod (8) remains constant.

The movement in the immersion fluid is very slight. Therefore a thorough aeration and mixing is necessary. This is accomplished by the device (3) shown in fig. 2. The small mica plate (4) attached to the aerating device serves the purpose of preventing excessive upward movements of the diver.

3. *The preparation of the sections*

For the permeability experiments plants were grown in waterculture as for the standard Avena test. This is briefly as follows:

Seeds were husked, soaked and laid out for 24 hours on moist filterpaper in a darkroom. Next, the germinated seeds were placed in glass holders, their roots in water. Two days later the plants were

ready for use. During the three days of cultivation the plants remained in the same darkroom at 23° C and 96 % relative humidity.

The procedure for cutting the sections has been described on page 390. The sections are infiltrated with water or a buffer mixture by centrifuging for 5 minutes at a rate giving the sections an acceleration of 500 times that of gravity. After infiltration the sections were kept in the darkroom for 5 hours in an amount of fluid corresponding to $2\frac{1}{2}$ ml per section and aerated. The composition of this solution can be varied according to the purpose of the experiments.

After 5 hours the sections were blotted carefully and 12 sections were placed for one hour in a small flask containing $\frac{1}{2}$ ml 50 % D_2O. During this hour the sections exchanged part of their ordinary water for heavy water. After 1 hour an equilibrium was established (BUFFEL, 1949) and the sections were lifted out from the flasks, blotted and placed on the divers, 12 sections per diver. The sequel of the experiment has been described in the preceding section.

The 50 % solution of heavy water was prepared through dilution from about 99,7 % D_2O. This dilution can be effected with distilled water, buffer mixtures or with solutions containing growth substances, according to the needs of the experiment.

Buffer mixtures were prepared from 1/100 mol.KH_2PO_4 and 1/100 mol.K_2HPO_4. For pH values below 4,5 1/100 mol.H_3PO_4 was added.

4.   *The influence of heavy water on growth*

In the years after the discovery of the heavy water many experiments have been carried out about the effect of D_2O on the activities of the living cell. The results differ. BARNES (1934) and MEYER (1934) found that *Euglena gracilis* and *Aspergillus* grew better in solutions containing small amounts of D_2O. LEWIS (1934) found that seed germination is impossible in pure D_2O and 50 % inhibited in 50 % D_2O. PACSU (1934) found that the alcoholic fermentation of d-glucose is retarded in about 100 % D_2O, while CALDWELL, DOEBELING and MANIAN (1936) found a higher inactivation of pancreatic amylase in about 100 % heavy water. LEWIS (1934) concluded on the ground of the experiments known to him, that D_2O is not very toxic, but only slows down the activities of the cell more or less in direct proportion to the % D on the total H present.

BUFFEL (1952) observed at the outset a stimulating effect of 50 % D_2O on the growth of *Avena* coleoptile sections with 1 % sucrose and 1 mg/1 I.A.A. After 12 hours in D_2O however the sections were flaccid.

WARTIOVAARA (1944) found that even concentrations of about 100 % D_2O apparently do not damage the cells of *Tolypellopsis stelligera*. In 50 % D_2O the cells remained alive for many days, while plasma rotation continued.

Many other investigations might be cited showing these phenomena. Part of these experiments has been carried out with inadequate techniques or the results are not supported by a sufficient number of observations. Many results, especially from the period immediately

after the isolation of heavy hydrogen, can be discarded because of insufficiently purified D_2O. In later years the quality of the D_2O preparations has been improved. Other investigators used freshly distilled heavy water. It is common knowledge that freshly distilled water contains much toxic mono- and dihydrol, which substances inhibit growth, while melted ice contains the much more favourable trihydrol (LLOYD and BARNES, 1932). The mechanism of the action of D_2O is not yet understood, but it is not necessarily a specific toxic action of the deuterium (FRERICKS, 1934). A slower uptake may also play a part.

We carried out some experiments in order to establish whether heavy water has an injurious effect on the sections in the permeability experiments. Therefore sections were placed in distilled water, 50 % and 100 % D_2O immediately after cutting. Ten sections were placed in small Petri dishes in 3 ml solution, no I.A.A. being added. The heavy water for this experiment had been distilled from alkaline permanganate some time before. Table 5 and fig. 4 show the results. D_2O appears to inhibit partly the autonomous growth. This is very evident in the highest concentration.
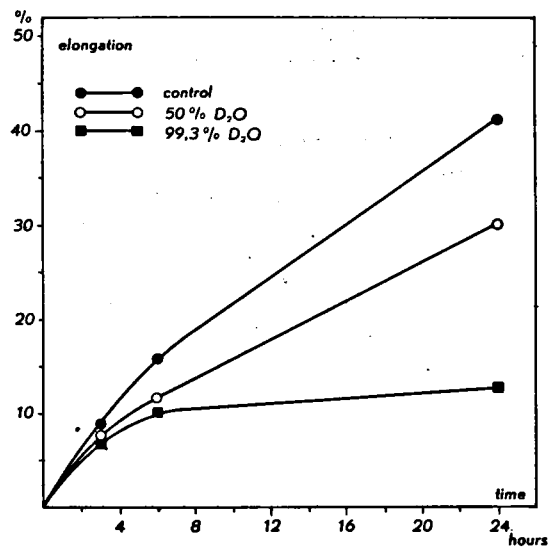

Fig. 4.   The effect of D_2O on the autonomous growth of sections.

TABLE 5
The growth of sections in heavy water

	% elongation		
	3 hours	6 hours	24 hours
Control	8,9	15,8	41,1
50 % D_2O.	7,6	11,6	30,2
100 % D_2O	6,8	10,0	12,6

For the permeability experiments it is important to know the effect of heavy water during the first hours after addition. The experiment described above is not conclusive in this respect. Therefore other experiments were carried out with 50 % D_2O only. This time the heavy water had not been redistilled. The sections were partly placed in heavy water immediately after cutting (fig. 5A), partly after a period of 5 hours in distilled water (fig. 5B). Measurements were made at short time intervals after the immersion in D_2O.
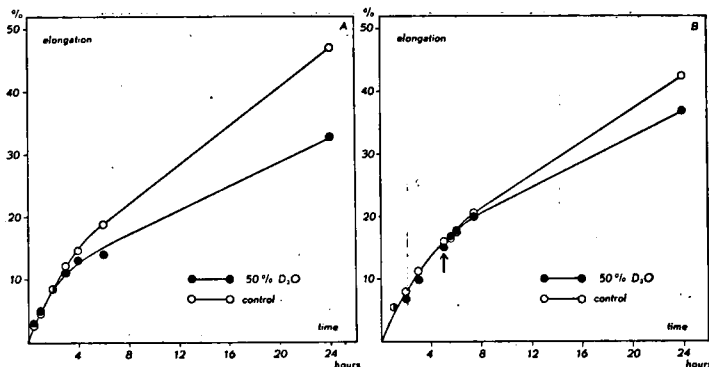

Fig. 5. The effect of 50 % D_2O on the autonomous growth of sections. A. Sections immersed in D_2O immediately after cutting. B. The arrow indicates the moment of addition of D_2O.

The typical experiment shown in fig. 5 again indicates an inhibiting effect, but this does not become evident before 3 or 4 hours after the immersion in D_2O. The final growth rate in D_2O is the same in both cases. For the permeability determinations we may neglect the injurious influence of D_2O, because the sections are in contact with D_2O for only 1 hour, while the damaging effect is not visible until 3 hours have passed after the addition of D_2O.

D. EXPERIMENTS

1. The effect of pH on the permeability to heavy water.

The effect of pH was investigated by treating sections with a buffer of a certain pH for some hours before determining the permeability. The time of treatment was varied so as to obtain a maximum effect. The permeability determinations were always carried out with the appropriate buffer mixture in the diffusion vessel. When the time of treatment is given as 0 hours, this means that the sections were not in contact with the buffer before they were placed in the diffusion vessel, which was then filled with a buffer of the stated pH.

Table 6 shows that no effect of the pH was found when the time of treatment was short (0–1 hours), while long periods of treatment with a low pH seemed to increase the permeability to heavy water. In table 6 a measure for the rate of diffusion of D_2O was introduced,

t_1, which is defined as the time taken by the concentration difference to fall to half its value. A decreasing t_1 denotes an increasing permeability.

TABLE 6

The influence of the time of treatment on the effect of pH on the diffusion of D_2O

Exp. no.	Time of treatment hours	t_1 min.		
		pH		distilled water
		4,17	5,8	
1	0	3,25		3,6
			3,4 .	3,4
2	1 6	4,75 2,25 2,25		4,0 4,25
3	½ 6	4,38 2,75		

A series of experiments was carried out in which the sections were treated with a buffer for 6 hours. Some complete diffusion curves, resulting from these experiments are shown in fig. 3 (page 404), while the t_1 values found are brought together in fig. 6. The trend line drawn through the points in fig. 6 must be considered as an arbitrary
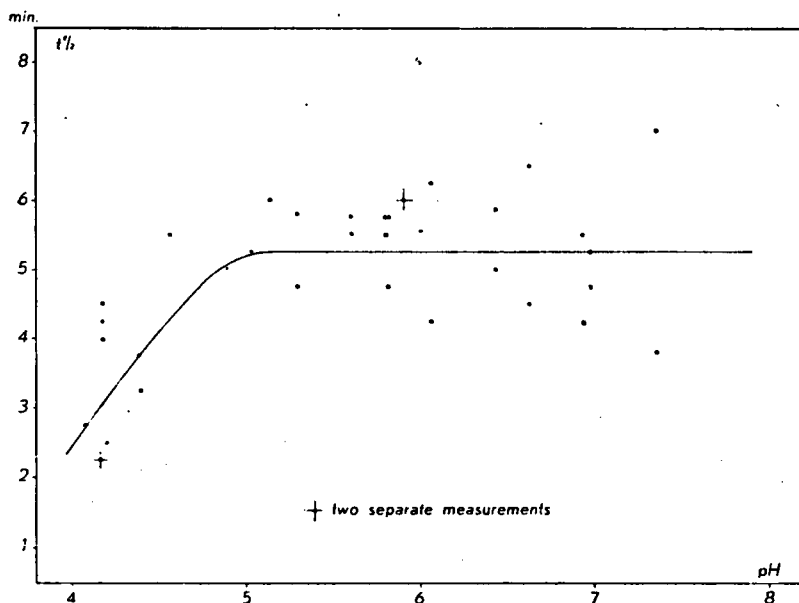

Fig. 6. The influence of pH on the permeability to heavy water.

approximation, for in the figure data are shown obtained at different days and at different times of the year. The variability of the reactions of coleoptile sections from one day to another is too well known to need any further emphasis. Therefore the values shown are not quite comparable.

Twenty different values of the pH between 4.08 and 7,36 have been tried. It is evident from fig. 6, that between pH 5,0 and 7,3 the permeability to heavy water remains constant, while it is definitely increased between 4,0 and 4,5. Compared with pH 6,9 for example generally 60–100 % increase is found. The exact position between 4,5 and 5,0 has not been established. This is very difficult because the small differences in this region cannot be measured significantly.

Three different causes can be held responsible for the modification of the permeability:

1. the difference of pH
2. the difference in the ionic composition of the solution.
3. the growth differences resulting from the different pH values.

1 and 2 cannot easily be separated, because pH and ionic composition change simultaneously. Growth differences, however, may be the cause of the differences in permeability, for a low pH stimulates the autonomous growth of the sections. This is shown in table 7.

TABLE 7

The effect of pH on the autonomous growth of coleoptile sections

pH	% elongation after			
	2 hours	4 hours	6 hours	24 hours
4,08	10,7	14,3	17,9	26,0
4,18	14,6	17,0	19,7	26,8
4,57	6,6	10,3	13,1	22,0
4,93	7,0	11,4	15,7	25,4
5,15	4,6	8,2	10,7	17,0
5,91	3,5	5,8	8,4	18,4

It appears from the table that during the 6 hours of treatment with the buffer mixtures a considerable amount of growth has taken place. Taking two extremes, pH 4,08 and 5,91, growth is twice as much in the former than in the latter. Assuming a constant diameter of the sections (this is approximately true, the increase in diameter being about 3 %), the increase in area will also show the proportion 2 : 1. The total area of sections treated for 6 hours with pH 4,08 is 1,09 times the area of the sections kept at pH 5,91. Nothing is known about the relation between elongation and a secondary increase in permeability, caused through this elongation, but even if it existed it does not seem likely that a difference in permeability of 60 – 100 % will be caused through a difference in area of only 9 %. Therefore, we are inclined to consider the difference found as an effect of the pH or the changed ionic composition of the buffer mixture.

2. *The effect of I.A.A. on the permeability to heavy water*

The influence of I.A.A. was investigated in the same way as the effect of the pH in the preceding section. Three different times of treatment were chosen: 0, 1 and 6 hours. The diffusion vessels always contained I.A.A. except in the case of the control determinations. As the optimum growth is found at a concentration of about 1 mg/1 I.A.A., only this concentration was used in these experiments. The results are shown in table 8.

TABLE 8

The influence of 1 mg/1 I.A.A. on the halving time ($t_{\frac{1}{2}}$) of D_2O diffusion from coleoptile sections

Treat-ment	$t_{\frac{1}{2}}$ min.										
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5 *		Exp. 6 *		Exp. 7 *		Average
Control	3,7	4,4	7,4	4,0	3,9	4,6	5,4		4,2	4,5	4,68
0 hours				4,0							4,00
1 hours	3,7	5,8	5,4	6,5							5,35
6 hours	4,6		5,4	5,4	5,2		5,2	4,8	5,1		5,10

* I.A.A. added in presence of 1/100 mol.KH_2PO_4. (pH 4.5)

No opening effect of I.A.A. on the ectoplasm was found in these experiments. With the exception of exp. 3 and 6 I.A.A. appeared to decrease the permeability to heavy water, as an increase in $t_{\frac{1}{2}}$ was found. The exception of exp. 3 may be left out of consideration, because the extremely high $t_{\frac{1}{2}}$ of the control was probably caused by some experimental error.

During the treatment with I.A.A. a considerable amount of growth took place. The control sections grew about 18 %, the sections treated with I.A.A. 35 %. This did not cause an increase in the permeability of the sections which showed the greatest elongation. During the permeability determinations some growth also took place, but the reduced weight remained constant within the experimental error. This could be expected, because the sections absorbed water only. When sections are observed for periods, much longer than 1 hour, a decrease of the reduced weight can be expected as a result of the loss of dry substance, which is used in respiration.

In contradiction with the findings reported in the preceding section it was found that the effect of I.A.A. was the same after 1 and 6 hours treatment. In both cases a slightly decreased permeability was found.

E. DISCUSSION

The experimental results described show very clearly that an increase in the permeability of the ectoplasm cannot initiate growth. The action of pH and growth substances will be localized somewhere else in the protoplasm. In respect to the action of I.A.A. these findings do not imply that the primary action of I.A.A. on the protoplasm is not a physico-chemical effect. As suggested by ANKER (1953), I.A.A. may affect lipophilic membranes in the inner part of the protoplasm,

Our experiments support to some degree a physico-chemical action of I.A.A., because they show that I.A.A. exerts an influence on membranes.

The results are in agreement with the findings of KONINGSBERGER et al. (1947), who also found a decrease in the permeability after the addition of I.A.A. They are at variance with the results of BUFFEL (1949, 1952), who found an opening effect on the ectoplasm. This difference may be caused through a difference in age and treatment of the sections.

The time relationschips of the action of I.A.A. seem to differ from those found by KONINGSBERGER et al. (1947), as no effect was found when the I.A.A. was added in the diffusion vessel only (0 hours treatment). In this respect our own data are insufficient, but BUFFEL (1949, 1952) and POHL (1948) also reported a treatment of at least about 1 hour to obtain any influence of I.A.A. This difference may be caused through a difference in material, KONINGSBERGER using isolated protoplasts, the other authors coleoptile sections.

The results of the experiments on the effect of pH on the permeability to heavy water are at variance with the data known in the literature. SEEMANN (1949) for example reports an optimum permeability at pH 6–7. Our data do not show an optimum at all. An explanation of this fact cannot be given yet. It may be a consequence of the difference in methods or material.

The increase in the permeability to water being excluded as cause of the higher growth rate under the influence of I.A.A., the osmotic properties of coleoptile sections will be examined in the next chapter.

Chapter III

THE OSMOTIC PROPERTIES

1. Introduction

It has been stated that a positive suction force must be maintained during growth. Therefore, the effect of growth substances may appear as an increase in suction force. As only a few data about the gross suction force of growing tissues under the influence of growth substances are available, it will be worthwhile to carry out determinations of the suction force. An enhancement of the suction force can be attained in two ways: a rise of the osmotic value of the cell contents, or a lessening of the wall pressure. A third possibility is the existence of an active water absorption, which may be promoted through growth substances. The following formula can be given for the suction force:

$$S = O + A - W$$

in which S means suction force, O osmotic value, A active water absorption and W wall pressure. S and O can be measured directly and this will be done in the experiments to be described in this chapter.

The object of these experiments is to examine whether the suction

force is enhanced through I.A.A. and to what extent changes of the osmotic pressure play a role in the reaction of coleoptile sections on I.A.A. Coleoptile sections are the preferable material for this purpose, because they react on growth substances immediately and grow in distilled water without any external supply of osmotic material, which simplifies the experimental conditions and the interpretation of the results.

Some definitions of the different terms used in this chapter run as follows:

The suction force is the force, expressed in atmospheres, with which water is attracted into a cell placed in distilled water. It is abbreviated as S.

The osmotic value is in numerical value equal to the potential maximum hydrostatic pressure which will develop in a solution if it is permitted to come to equilibrium with pure water in an ideal osmotic system (MEYER, 1945). The osmotic value is expressed in atmospheres and abbreviated as O.

The wall pressure is an inwardly directed hydrostatic pressure, expressed in atmospheres, originating as a reaction of the elastically stretched cell wall. It is abbreviated as W.

The turgor pressure is the outwardly directed hydrostatic pressure of the cell contents on the cell wall. It is abbreviated as T and also expressed in atmospheres. In tissues, T and W are not necessarily equal, as tissue tension or other external pressures may exist which help to counterbalance the turgor pressure. In this case turgor pressure is equal to wall pressure plus external pressure (P):

$$T = W + P$$

2. LITERATURE

The data, which can be gathered from the relevant literature, show that generally the osmotic value of growing tissues remains constant, or decreases only slightly, when a source of osmotic active material is present (e.g. the seed or salts or sugars in the external solution). The amount of osmotically active material per cell increases markedly. These phenomena have been investigated extensively by their discoverer PFEFFER (1893) and by URSPRUNG and BLUM (1924). The latter also measured the suction force in the roots of *Vicia Faba* and found that the suction force is maximum in the zone of maximum elongation. An extensive review of the older literature was given by HEYN (1931, 1940).

Fewer data are available about the influence of growth substances on S and O. RUGE (1937) found a drop in the osmotic value of I.A.A.-treated *Helianthus* hypocotyls. DIEHL et al. (1939) found that the osmotic value in epidermal cells of *Helianthus* hypocotyls treated with I.A.A. was smaller than in the control series. They used, like RUGE did, hypocotyls still attached to the seeds and with a developed root system.

BURSTRÖM (1942) found an increase in osmotic active substances induced by I.A.A., while the osmotic value during growth was dependent on the nutritional condition of the roots.

OVERBEEK (1944), LEVITT (1948) and HACKETT (1952) showed that the osmotic value of potato tuber discs is decreased after I.A.A. treatment (see Ch. I, par. 2).

Data about the influence of growth substances on the suction force are very scarce. RUGE (1937) stated that S decreased after treatment of hypocotyles with I.A.A., while BRAUNER and HASMAN (1949) found that the suction force of I.A.A.-treated potato tuber discs has fallen below that of discs kept in water after 6 hours exposure. After longer exposure times, however, the suction force is greater in the treated discs, in spite of the greater amount of water absorbed. The differences are very small, however.

So far it seems to have been established that I.A.A. does not enhance the osmotic value, while the data about the suction force are conflicting. Moreover, a drawback of these investigations is that generally, S and O have not been determined for the same material and in the same experiment.

3. METHODS

The "simplified method" of URSPRUNG and BLUM (1923) allows the determination of the suction force by measuring changes of length in solutions with different osmotic values. For the determination of the osmotic value the external concentration at which incipient plasmolysis occurs must be known. Incipient plasmolysis is very hard to observe in tissues. However, when tissues are placed in solutions with higher osmotic values than those used in the suction force determinations maximum shrinkage will occur at a certain osmotic value of the external solution. In other words, the length of the tissues becomes constant within the experimental variation, independently of the hypertonic external osmotic value. This means that the tissue is plasmolysed and has no turgor. The first concentration, which gives the constant length, can be considered as the concentration of incipient plasmolysis. Of course this is an approximation, which will be the more accurate, as the concentration differences employed are smaller. When the initial length and the length at incipient plasmolysis are known, the osmotic value can be calculated.

The above principle has been applied in this investigation as follows: 10 sections, prepared as described on page 390, were mounted on a slide and placed in a Petri dish. A number of parallel series was prepared. As osmotic active agent mannitol was chosen, because it permeates very slowly and does not support growth. Different molar concentrations were prepared and each series was measured and placed in a different concentration. 50 ml solution was used for 10 sections. The concentrations varied between 0 and 1,0 mol. in 1000 grams H_2O. After 1 hour the sections were measured again and the changes of length were calculated. The values found are

brought together in a graph, which shows the change in length as a function of the change in concentration or osmotic value of the external solution. The point of intersection of the graph and the abscissa shows the value of the suction force, while the point at which the graph becomes horizontal (always below the abscissa) denotes the concentration of incipient plasmolysis. This method is referred to as method I.

The results obtained with the coleoptile sections give a crude approximation of the order of magnitude, as it is not possible to obtain reliable results with concentration differences smaller than 0,05 mol. This is a difference in osmotic value of about 1,1 atm. Smaller differences cannot be employed due to the variation in reaction between the sections.

Of course it is possible to determine suction force and osmotic value after various times of treatment with growth substances. But the number of treatments to be tested in one experiment is limited, as at least 8 to 10 series of ten sections are needed for every determination of suction force, plus osmotic value. It will be found desirable to use 11 or 12 series, which allow the use of more different concentrations of the mannitol solutions with small grades, especially near the expected values of S and O_i (O at incipient plasmolysis). In addition to the treated series a control series must be run, so that in all, at least 20 series of 10 sections must be prepared. This limits the number of treatments which can be examined in one experiment. For the determination of the suction force 5 series is the minimum number.

Instead of measuring the differences in length, it is possible to determine the changes in water content in solutions with a different osmotic value by weighing the sections before placing them in the solutions, and after one hour's submersion. This method is referred to as method II. It allows the determination of the suction force only, as it is not possible to determine the point of incipient plasmolysis in this way. The results are somewhat more exact than those obtained by measuring the changes of length.

4. THE EFFECT OF I.A.A. ON THE OSMOTIC PROPERTIES

To start with, S and O were determined immediately after cutting the sections and after some hours' submersion in distilled water. One

TABLE 9

Suction force, osmotic value and wall pressure of coleoptile sections immediately after cutting and after some hours in distilled water

Exp. no	Time of treatment in dist. water hours	S atm.	O atm.	W atm.
1	0	8,63	—	—
2	0	6,93	12,72	5,79
3	5	2,60	10,50	7,90
4	6½	3,58	10,62	7,04
5	5½	2,65	12,78	10,13

of these experiments is presented in fig. 8, while the data are brought together in table 9.
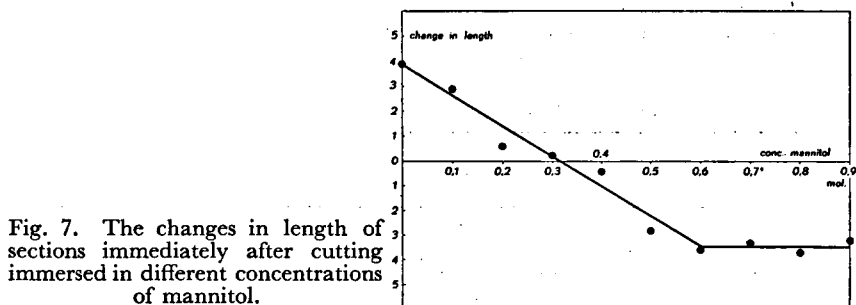

Fig. 7. The changes in length of sections immediately after cutting immersed in different concentrations of mannitol.

It appears from the table that S is very high, 7–8 atm., immediately after cutting and drops rather fast in distilled water. Assuming that no active absorption of water occurs (this will be discussed later)

$$S = O + A - W$$

is simplified to

$$S = O - W$$

From this W can be calculated. The results are compiled in the last column of table 9. W appears to be fairly high in the coleoptile and shows a tendency to increase during the immersion of the sections in distilled water.

After establishing these facts the suction force of $7\frac{1}{2}$-mm sections was determined by using method II. The sections were treated with
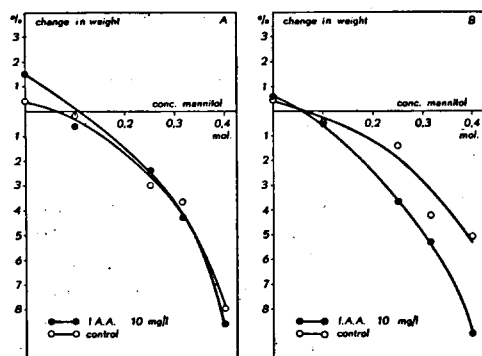
Fig. 8. The effect of I.A.A. on the suction force. A. After $3\frac{1}{2}$ hours treatment with I.A.A. B. After $27\frac{1}{2}$ hours treatment with I.A.A.

I.A.A. before the determinations were carried out. The results are shown in table 10, while experiment 3 is presented in fig. 8. From these results it is clear that S must be increased by I.A.A., while the increase is not found after a long time of treatment.

To examine the osmotic value a more extensive series of experiments was carried out by using method I. Before determining S and O the sections were treated with I.A.A. 5 hours was chosen as the most

appropriate time of treatment. The results are given in table 11 and a typical experiment is shown in fig. 9. Table 11 shows that after 5 hours of treatment with I.A.A. the suction force is not greater evidently than in the control series. Considering the experiments 1

TABLE 10

The effect of 10 mg/l I.A.A. on the suction force of $7\frac{1}{2}$ mm coleoptile sections

Exp. no.	Treatment	$S_{I.A.A.}$ atm.	$S_{Contr.}$ atm.
1	5 hrs. dist. H_2O, 1 hr. I.A.A........	2,71	1,58
2	$2\frac{1}{2}$ hrs. I.A.A...............	4,07	3,36
3	24 hrs. dist. H_2O, $3\frac{1}{2}$ hrs. I.A.A......	2,46	1,34
	24 hrs. dist. H_2O, $27\frac{1}{2}$ hrs. I.A.A......	1,12	1,12

up to and including 8 and leaving the differences in I.A.A. concentration out of account, it appears that $S_{I.A.A.}$ and $S_{contr.}$, are the same in 6 cases, $S_{I.A.A.}$ is greater in one case and smaller in 3 cases (differences smaller than 0,5 atm. have been neglected). After shorter times of
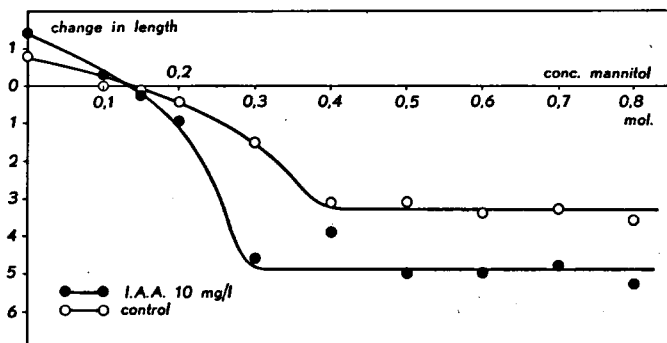

Fig. 9. The effect of 5 hours treatment with 10 mg/1 I.A.A. on the suction force and the osmotic value at incipient plasmolysis. The change in length is given in scale units of the eyepiece micrometer used in the measurements.

treatment, however, $S_{I.A.A.}$ is nearly always higher than $S_{contr.}$ (compare table 10). Fig. 12 (page 427) shows that the growth rate is maximum during the first hours after the addition of I.A.A. After 5 hours nearly half of the total elongation to be found after 24 hours has taken place. The growth rate of I.A.A.-treated sections is still higher than that of the control sections, but much lower than immediately after the addition of I.A.A. This may offer an explanation for the negative results obtained after prolonged treatment with I.A.A. The fact that no significant increase in S is found after 5 hours does not exclude definitely the existance of an increase. The method used is not very accurate and small differences will escape from observation.

The results of the determinations of O give more clear-cut results. In all experiments $O_{I.A.A.}$ is lower than $O_{contr.}$. This is in accordance with the data reported in the survey of the relevant literature. The

possibility of osmotic material being formed in the sections through hydrolysis of higher carbohydrates (starch being the most likely source) cannot be rejected with the argument that the osmotic value does not increase. Only direct determinations of the total amount of sap solutes, or a determination of the product of osmotic value and volume (VAN 'T HOFF's law), can give information about this

TABLE 11

The effect of I.A.A. on suction force and osmotic value. I.A.A. was added immediately after cutting

Exp. no.	Time of treatment hours	Conc. I.A.A. mg/l	S atm.		O atm.		W — A atm.	
			I.A.A.	Contr.	I.A.A.	Contr.	I.A.A.	Contr.
1	5	10	1,15	2,33	6,70	9,63	5,55	7,30
2	5	10	1,90	2,20	6,04	9,60	4,14	7,40
3	5	10	2,56	1,40	7,32	7,46	4,76	6,06
4	5	12,2	2,31	2,19	6,01	8,44	3,70	6,25
5	5	10	2,84	2,94	6,08	8,41	3,24	5,47
6	5	10	2,45	3,31				
		10	2,70		8,12		5,42	
7	5			2,50		8,42		5,92
		1	2,60		8,19		5,59	
8	5	1	2,63	2,51	8,21	8,57	5,58	6,06
† 9	5	1	1,87	2,60	7,26	7,51	5,39	4,91
		1	2,20					
*10	1			2,20				
		10	2,70					
*11	1	10	4,20	2,82				

† The control sections showed a higher growth rate after the determinations of S and O than the I.A.A. series.
* The sections were kept in distilled water for 5 hours after cutting, then I.A.A. was added.

problem. If no osmotically active material is formed the product mentioned must be approximately the same for control and for I.A.A.-treated sections. If, on the other hand, I.A.A. causes a stimulation of the formation of osmotically active substances the product must be higher in the presence of I.A.A. The available experimental data for the experiments listed in table 11 allowed an approximate calculation of the product. Table 12 shows the values of the product found in a number of experiments. As the diameter of the sections is nearly constant, the length of the sections was substituted for their volume. The results of the calculations are given in table 12. The product $O \times L$ shows a tendency to decrease under influence of I.A.A. This means that I.A.A. has no effect on the formation of osmotic material. Hydrolysis of starch may be promoted by I.A.A., but in that case the resulting products are entirely consumed in the metabolism.

From the values found for the osmotic value it can also be concluded that, although the osmotic value of the I.A.A.-treated sections is smaller, both I.A.A.-treated and control sections have about the

TABLE 12

The effect of 5 hours treatment with 10 mg/1 I.A.A. on the product osmotic value × length

Exp. no.	$O \times L$	
	I.A.A.	Control
1	349	432
2	316	426
3	362	326
4	312	371
5	313	376
7	397	381

same suction force. This indicates that I.A.A. affects another component of the suction force. As no proof is available that an active absorption of water promoted by I.A.A. does not exist, this possibility must be taken into account. The formula denoting the suction force was stated to be

$$S = O + A - W$$

This can be written as

$$O - S = W - A$$

As O and S are known, the difference $W - A$ can be calculated. The calculated values can be seen in table 11. Indole-3-acetic acid decreases the difference $W - A$. Thus W or A may be changed, W being decreased or A being increased. A choice between these two possibilities cannot be made, but an indication may be found in fig. 9 (page 416), which shows that I.A.A.-treated sections shrink more during plasmolysis than do the control sections.[1] Consequently, the treated sections are stretched elastically to a greater extent in turgescent condition, which implies a greater elasticity of the cell wall, as the sections immersed in water are always fully turgescent. Thus I.A.A. may influence the wall properties and in this way change the suction force.

5. DISCUSSION

Although no evident change in the magnitude measured as the suction force S has been found after longer times of exposure to I.A.A., probably because of methodical limitations, no doubt is left

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[1] In fig. 9 the changes in length are given after the immersion of the sections in mannitol solutions. The initial length (before the immersion in mannitol) will be greater in the I.A.A.-treated sections than in the control sections, for I.A.A. will have promoted elongation. In the experiment shown in fig. 9 the above-mentioned initial length of the control sections was 44,2 units and that of the I.A.A.-treated sections was 52,1 units. The length immediately after cutting the sections was 39,8 and 39,7 respectively. The elastic extension was 3,3 and 4,9 respectively. These data demonstrate that the irreversible increase in length is greater than the increase in the reversible part of cell length after the addition of I.A.A.

about the fact that growth substances affect the suction force. It has been proved that the osmotic value of the cell contents decreases with increasing elongation, while the suction force remains more or less constant. The osmotic value is lowest in sections treated with I.A.A. and the value of the product $O \times L$ indicates that I.A.A. does not increase the total quantity of sap solutes. But even if the osmotic value remains constant during the elongation, this cannot be an explanation for the higher growth rate, which is maintained in I.A.A., for the wall pressure would increase very quickly and would prevent any further growth. The same argument is valid for the possible effect of I.A.A. on an active process of water absorption.

Therefore, a change in wall properties seems to be necessary, the more so as both plastic and elastic stretching of coleoptile tissue are rather limited. A change in the elastic properties of the wall was indicated in the reported experiments, as has been pointed out on page 418. This is a fairly direct proof. More indirect evidence can be found in the experiments of table 10. In fig. 8 it focusses the attention on the fact that both in figure A and figure B the graph representing the changes of weight of the I.A.A.-treated sections is less concave towards the abscissa than the one representing the control. HASMAN (1943) and BRAUNER and HASMAN (1949) pointed out that the positive part of the S-curve (above the abscissa) will be less concave as the tensility of the cell wall is higher. These experiments support therefore an effect of I.A.A. on the cell wall. This will be investigated more in detail in experiments to be described in the next chapter.

It must be emphasized that the suction force that is found after prolonged immersion in distilled water or in I.A.A., originates from growth and that it cannot be accounted for osmotically. This fact should be well borne in mind. A simple calculation in using the formula $S = O - W$ will not give any information about the origin of this suction force, since the unknown active component resulting from growth always plays a part.

An estimate of the magnitude of this active component is possible, when it is assumed that the sections are fully turgescent after some hours' (3–5 hours) immersion in distilled water. This assumption is justified by the fact that in osmotic studies it takes fairly short times to establish an equilibrium. If the assumption is correct the wall pressure will be equal to the osmotic value in numerical value. In that case the active component of the suction force will be equal to the suction force found. It is therefore likely that the suction force measured after prolonged treatment with distilled water or with an I.A.A. solution must be ascribed to growth alone.

CHAPTER IV

THE ELASTIC PROPERTIES OF THE CELL WALL

1. INTRODUCTION

The experimental results described in the preceding chapter suggested an effect of I.A.A. on the elastic properties of the cell wall. The experiments to be reported here were carried out to provide this hypothesis with more evidence, in order to establish the time relationship of the possible effect of I.A.A. on the wall and to compare the effect of I.A.A. on the cell wall with its effect on the growth rate. First a survey of the literature on the subject will be given.

2. LITERATURE

In view of the great number of publications available it is not possible to give a complete review in the limited space available. This survey will therefore be restricted to the effect of growth substances on the elastic properties of the cell wall.

HORREUS DE HAAS (1929) suggested a relation between growth substances and wall properties. He found that the elasticity is higher in the upper side of geotropically stimulated roots of *Vicia Faba* than in the lower side. Decapitated coleoptiles were less elastic than intact coleoptiles.

HEYN (1931) made extensive investigations into the effect of decapitation on the wall properties of *Avena* coleoptiles. Decapitation lowered elastic and plastic extensibility, while the extensibility increased again after the application of growth substance. HEYN concluded that the elasticity has no importance at all for growth but that it is increased as a result of elongation. Growth substances would regulate growth via the plasticity of the cell wall.

HEYN and VAN OVERBEEK (1931) determined the plastic and elastic extensibility of coleoptile sections. Both are increased by growth substances. The increase in elastic extensibility is rather considerable.

SÖDING (1931, 1932, 1934) found that elastic and plastic extensibility and growth rate are interconnected and also show a relation with the amount of growth substance available. The turgor extension is increased after treatment of *Avena* coleoptiles with growth substance for $1\frac{1}{2}$ hour. SÖDING considered the increase in both plastic and elastic extensibility as a result of the elongation and as an accompanying phenomenon.

RUGE (1937) found that the elastic extension of *Helianthus* hypocotyls was increased after 18–24 hours treatment with concentrated I.A.A. pastes. RUGE postulated that this increase originated as a result of the decrease in wall thickness during elongation. The author states that the wall properties of plasmolysed and dead cells are also changed by I.A.A. His experiments, however, are liable to critical consideration (see page 430).

DIEHL et al. (1939) treated *Helianthus* hypocotyls with lanoline

pastes containing I.A.A. They found a maximum elastic extensibility after 2 hours treatment. The wall properties were changed after 1 hour treatment with I.A.A., the change is maximum after 2 hours and is maintained for some hours.

GESSNER (1936) observed that during phototropic stimulation the shadow side showed an extremely high extensibility. Light decreased both elastic and plastic extensibility in *Helianthus* hypocotyls. The author ascribes these changes to assumed changes in the concentration of the growth substances in the tissue after illumination.

AMLONG (1936) found that the roots of *Vicia Faba* grew faster after decapitation, but the elastic and plastic extensibility were smaller after decapitation. This implies that there is no direct relation between wall properties and growth rate. The different concentration for growth substance action on roots and stems cannot be explained through an effect on the cell membrane. AMLONG (1939) found that low concentrations or growth substances promoted growth rate, plastic and elastic extensibility. The optimum concentration is the same for all three.

The results of BURSTRÖM (1942) are partly at variance with those of AMLONG, as BURSTRÖM found that inhibiting concentrations of I.A.A. still increased the elastic extension and no joint optimum could be established. BURSTRÖM states that the increase in extension precedes elongation and is important as an initiator of growth. FREY WYSSLING (1948, 1952) used some of BURSTRÖM's data in calculations and obtained the result that the Young's Modulus decreases most markedly during the early phase of elongation, but rises again steeply without an obvious decrease of the growth rate. The author considers the increased elasticity as an essential part of the elongation process.

From these results it appears that, generally speaking, growth substances effect a rise in the elastic properties of the cell wall, determined as elastic extension or as elastic extensibility. Most of these authors reject the idea that the elastic properties occupy a key position in the elongation process, while some of them assume that an increased elasticity is important in the starting phase of growth.

3. METHODS

Sections were prepared according to the procedure described on page 390. These sections were treated with I.A.A. under the usual conditions for the section test (page 390). After some time the length of the sections was recorded and they were removed from the solution containing I.A.A. and plasmolysed with 1 mol. mannitol (this means 1 mol. mannitol solved in 1000 grams water). After 1 hour in the mannitol solution the sections were measured again. The difference between the length of the turgescent cells (L_t) and the plasmolysed cell (L_p) was calculated. This difference (dL) was called elastic extension. Thus:

$$dL = L_t - L_p$$

A change in elastic extension was regarded as an indication of a change in the properties of the cell wall. The extension was noted in scale units of the eyepiece micrometer used in the measurements. One unit corresponds to 60μ.

To attain a relation with the length of the sections we introduced a factor E, which was defined as:

$$E = dL/L_p \times 100.$$

The comparison of E-values comes to the comparison of the average elasticity of the corresponding sections. For a discussion of E see page 439.

4. THE INFLUENCE OF I.A.A. ON THE ELASTIC EXTENSION

In a series of experiments sections were treated with I.A.A. for about 5 hours. The I.A.A. was added immediately after cutting. The elastic extension was determined and compared with the extension of a control series. The results are brought together in table 13.

TABLE 13

Elastic extension after 5 hours treatment with I.A.A.

Exp. no.	Elastic extension scale units		E %		Number of series and of sections		Conc. I.A.A. mg/l
	I.A.A.	Control	I.A.A.	Control	I.A.A.	Control	
1	5,6	3,4	12,0	8,3	9 × 10	6 × 10	10
2	5,3	3,5	11,3	8,6	7 × 10	5 × 10	10
3	5,2	3,3	11,9	8,0	4 × 10	4 × 10	10
4	3,7	2,5	8,1	6,1	5 × 10	5 × 10	10
5	4,4	3,2	9,2	7,7	6 × 10	4 × 10	12,2
6	4,8	3,3	10,3	8,1	6 × 10	5 × 10	10
7	6,6	3,6	13,4	8,5	1 × 10	1 × 10	10
8	5,3	2,7	10,7	6,1	2 × 10	2 × 10	1
9	4,8	3,3	9,3	8,0	4 × 10	4 × 10	1
10	4,2	2,2	8,5	5,2	5 × 10	5 × 10	1

The table shows that in all these experiments the elastic extension is higher after treatment with I.A.A. This increased extension indicates very clearly that the final effect of I.A.A. is found in the cell wall, but it does not prove that the primary reaction of the growth substance is also located in the wall. It can only be said that in general fast growing cells have a greater elastic extension than slowly growing sections.

The concentrations used in these experiments all have a promoting influence. The increase in elastic extension is for both concentrations in the same order of magnitude. From other experiments it may be concluded that in one and the same experiment 10 mg/1 I.A.A. has a greater effect than 1 mg/1. A more systematic investigation of this phenomenon has not been made.

All determinations mentioned in table 13 were carried out after about 5 hours treatment with I.A.A. For this reason it seemed desirable

to investigate the time sequence of the increase in elastic extension and to compare this with the growth reaction. This was accomplished by adding I.A.A. to a number of series and determining the elastic extension at different times after immersion in the solutions. Table 14 shows results with sections which were treated immediately after cutting. After 5 hours treatment the increase in elastic extension is

TABLE 14

Elastic extension in relation to the duration of the treatment with I.A.A. [1]

Time of treatment hours	Elastic extension scale units		E %		Conc. I.A.A. mg/1
	I.A.A.	Control	I.A.A.	Control	
0		4,4		11,8	
1	5,4	5,0	13,7	13,0	
5	7,4	2,9	14,4	6,8	10
24	6,7	2,8	11,6	6,1	

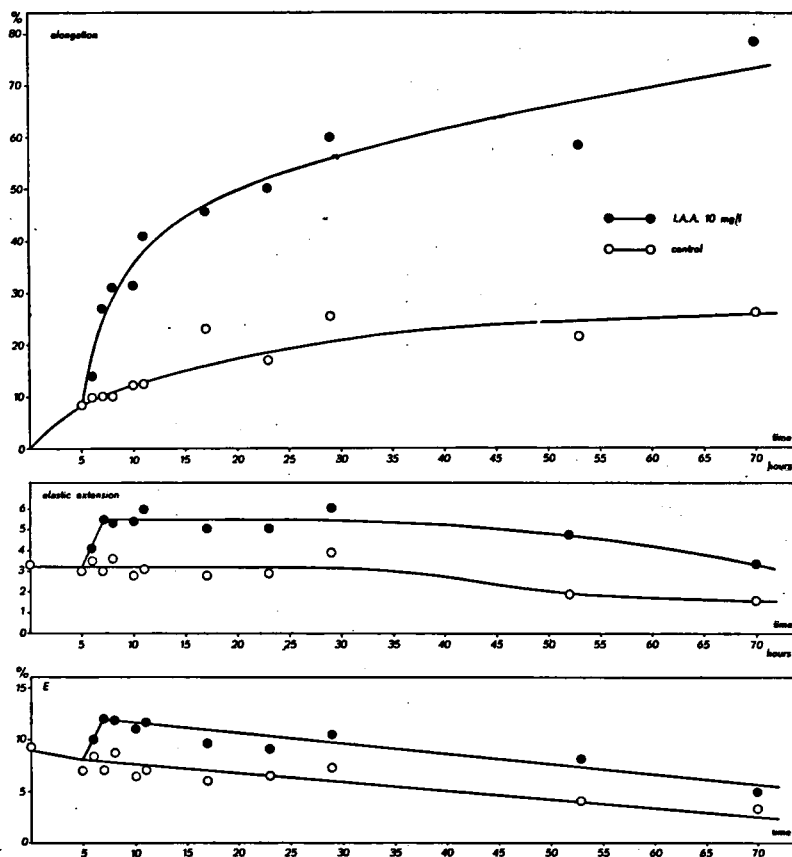[1] Every value was determined from 1 series with 10 sections

Fig. 10. The effect of 10 mg/1 I.A.A. on elongation, elastic extension and E.

considerable, especially when one compares the absolute values. After 1 hour, however, there is no significant difference with the control.

This problem was investigated more extensively with sections treated with I.A.A. after immersion in distilled water for 5 hours after cutting. The results are collected in table 15.

The experiments 3 and 5 indicate that the first 2 hours are very

TABLE 15

Elastic extension in relation to the time of treatment with indole-3-acetic acid. The sections were immersed in distilled water during 5 hours before treatment

Exp. no.	Time of treatment hours	Elastic extension scale units		E %		Conc. I.A.A. mg/1
		I.A.A.	Control	I.A.A.	Control	
1	0		3,1		7,5	
	¼	3,0	3,0	7,2	6,9	
	½	3,5	3,5	8,7	8,5	
	1	3,8	3,3	8,9	8,3	10
	1½	4,8	3,0	11,5	7,3	
	2	5,1	3,0	12,1	7,2	
2	0		3,0		7,0	
	1	4,1	3,5	9,7	8,6	
	2	5,5	3,0	11,9	7,1	
	3	5,3	3,6	11,8	8,7	
	5	5,4	2,8	11,0	6,6	
	6	6,0	3,1	11,7	7,1	10
	12	5,2	2,8	9,6	5,9	
	18	5,2	2,9	9,1	6,5	
	24	6,1	3,8	10,3	7,8	
	48	4,8	1,9	8,1	4,0	
	65	3,4	1,6	4,8	3,2	
3	0		3,6		8,6	
	1	4,6	3,7	10,3	9,0	
	2	5,9	2,5	13,1	6,0	
	19	7,2	3,1	11,3	6,5	10
	24	6,2	4,3	9,2	8,8	
	41	7,8	2,6	11,0	4,8	
4	0		2,8		6,3	
	½	4,0	3,1	9,6	7,3	
	1	4,5	3,3	10,2	7,7	1
	18	8,3	3,9	12,7	5,9	
5	0		3,8		9,3	
	1	4,1	3,7	9,5	9,1	
	2	4,9	3,4	10,9	8,0	1
	3	5,2	3,2	10,9	7,7	
	21	6,0	3,1	9,9	6,7	
6	1	4,6	3,7	10,8	8,8	10
7	1	4,6	3,6	10,4	8,3	1

important for the reaction of the elastic extension on the addition of I.A.A. It seems that the maximum value of E is reached after 2 hours, while the extension in absolute measure still increases. Lack of data between 2 and 20 hours after the addition of I.A.A. made these results less valuable. Experiment 2 was therefore carried out (fig. 10). Elongation, extension and E were determined at different times. The fastest growth takes place during the first hours after the addition of the growth substance. The elastic extension reaches its maximal value after 2 hours and remains more or less constant until 24 hours after the immersion in the I.A.A. solution. After 24 hours it diminishes perceptibly. As the extension remains the same and the length of the sections increases, the E-values decrease from the second hour. This is shown in the lower graph in fig. 10.

Not every experiment showed a constant elastic extension after 2 hours. This can be seen from experiment 3 for example. Here the values found after 19 and 41 hours are much higher than the extension after 2 hours. This frequently occurs and will also be found in the experiments described in the next chapter. However, the extension increases more slowly after 2 hours, than from 0 to 2 hours. Generally, this effect will not be found when using E as an indicator. Then a decrease is seen from the second hour.

Another interesting question is: when does the first measurable reaction occur? Experiment 1 shows that it takes at least 1 hour before any reaction can be detected, while the greatest increase appears between 1 and 1½ hours after adding I.A.A. This is also illustrated by data from table 16. For this table the same experiments have been used as in table 15. Sometimes an appreciable promotion of

TABLE 16
Elastic extension after auxin treatment in % of the control

Time hours	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6	Exp. 7
¼	100						
½	100			129			
1	115	117	125	132	111	124	128
1½	160						
2	170	184	184		149		
3		148			163		

the elastic extension is found after 1 hour, but a much greater effect is always found between 1 and 2 hours after the addition of I.A.A. This is shown very clearly in fig. 11. Here the ratio $E_{I.A.A.}/E_{contr.}$ has been used for comparison. The ratios found in this case are somewhat different from those derived from the elastic extension, but the general trend is the same.

It is desirable in view of the above stated results, to examine the growth reaction during the first hours after addition of growth substance. This study might be realized with data from the experiments shown in table 15, for example exp. 2 (fig. 10), a procedure, less

advisable, because in that case every growth rate determination is made on a different series of sections. It may cause great differences in the case of growth measurements at short time intervals. We therefore used for this analysis growth curves of one series of sections (fig. 12).

The broken lines in fig. 12 were determined with sections which were immersed in distilled water before I.A.A. was added. For about
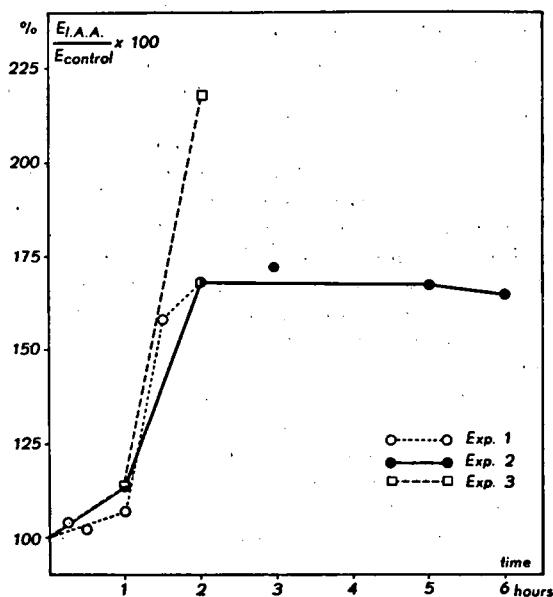

Fig. 11. For explanation see text.

5 hours after the addition of I.A.A. the growth rate will virtually be the same, as is indicated in fig. 12 by the constant slope of the growth curve. Thus about a linear relation exists between time and elongation during this time.

TABLE 17

The growth rate of isolated sections (kept for 5 hours in dist. water first) during the first hours after the addition of I.A.A. SD.: standard deviation

Conc. I.A.A. mg/l	Growth rate % elongation/hour								
	Exp. 1			Exp. 2					
	1st hr.	2nd hr.	3rd hr.	1st hr.	SD.	2nd hr.	SD.	3rd hr.	SD.
0	1,2	0,9	0,9	2,2	1,06	1,4	0,70	1,6	0,52
0,01				3,8	0,92	2,9	0,57	3,1	0,36
0,1				3,9	0,88	2,9	0,57	3,2	0,79
1	7,1	7,8	7,4	2,7	0,68	2,9	1,10		
10	4,9	4,8	4,7						

This is confirmed by the data shown in table 17. Exp. 1 in the table is the same as the one shown in figure 12. The data given in table 17 for exp. 1 have been partly estimated from the growth curves. In both experiments the sections used for the analysis of the growth rate were treated with distilled water for about 5 hours after cutting
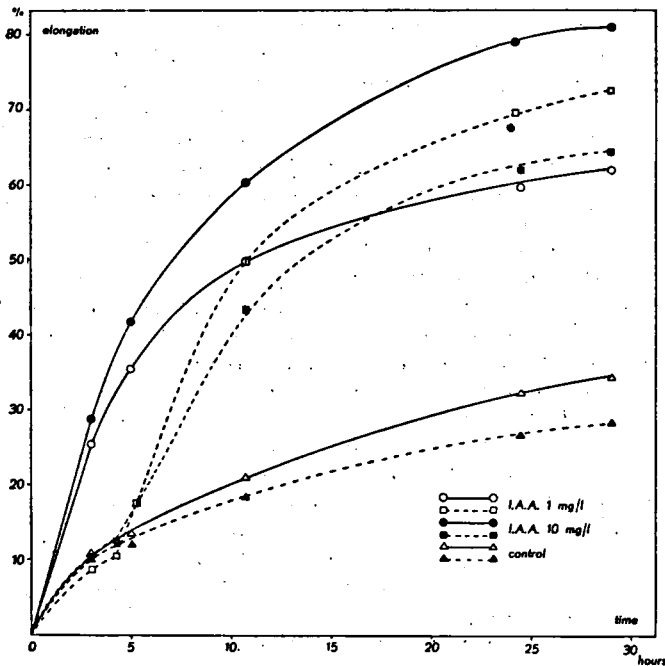

Fig. 12. Growth curves of sections treated with I.A.A. and of control series. The solid lines are determined with sections immersed in I.A.A. immediately after cutting. For the broken lines sections were pretreated with distilled water for 4½ hours.

them before the I.A.A. was added. The table shows that the growth rate remained constant within the limits of the experimental error during 3 hours after the addition of I.A.A. This was also found for different concentrations of I.A.A. The growth rate reaches its maximum value in the first hour, without a visible lag. This behaviour is quite different from the facts found with the elastic extension. There seems to be no parallel between the response of the growth rate and the elastic extension to the external addition of indole-3-acetic acid.

5. DISCUSSION

Growth substance added to isolated sections of the *Avena* coleoptile causes changes in growth rate and in elastic extension. The time sequences of these changes are not identical. The growth rate increases immediately after the addition of I.A.A. and remains constant for

several hours. The results of determinations of the elastic extension seem to indicate a time lag, while the extension does not increase any more — or only very slowly — after 2 hours exposure to I.A.A. This might suggest that no direct relation exists between growth rate and elastic extension.

However, some objections must be considered. In the first place the growth reaction may show a time lag too, but a rather short one, a fact very difficult to discover owing to the variability of the material and the error of the measurements, especially as very small differences are involved. It does not seem very likely, however, for GAST (1942) found that roots of maize seedlings immediately responded to I.A.A., after they had been in contact with the growth substance for only two seconds. RIETSEMA (1950) also found that I.A.A. brings about a rapid increase in growth rate.

Another possibility is that the time lag of the response of the extension is shammed by the limitations of the method. This does not seem very likely either. A minimum change must be effected indeed before it can be measured with the method used. Assuming a constant rate of increase in extension, no increase can be expected during the first measurements, for instance after $\frac{1}{4}$ and $\frac{1}{2}$ hour. If the extension would change at a constant rate the effect after one hour would be about half of the total increase to be found after two hours. This is of a magnitude that might be detected in the section test. If this assumption were true, a rapid increase ought to be found between $\frac{1}{2}$ and 1 hour after the addition of I.A.A. and a proportionally slower increase between 1 and 2 hours. This does not hold true.

For this reason we are inclined to consider the difference in time sequence between elongation and extension as a real and important phenomenon. Apparently the elastic extension is not directly connected with the elongation, but the change in extension may indicate a fast growing of the primary wall without any appreciable apposition of secondary layers (see page 439). In that case the increase in elastic extension is an accompanying phenomenon.

CHAPTER V

THE MECHANISM OF THE ACTION OF GROWTH SUBSTANCES ON THE CELL WALL

1. INTRODUCTION

It appeared from the data given in the preceding chapters that growth substances influence the cell wall. The mechanism of this action has been passed by in silence, so the following part of this investigation is engaged with this problem. In the literature on the subject different opinions have been presented, but a settled explanation is not yet available. This will appear from the selection of the relevant literature in section 2.

The main problem will be to discriminate whether the action of

growth substances is a direct one or whether the changes in wall properties are effected through the intermediary of the protoplasm. Two main ways are open to approach this object:

1. An attempt to destroy the structural relation between protoplasm and cell wall, e.g. by plasmolysing or killing the cells.
2. An attempt to influence the wall properties with substances, which affect protoplasmic activities (growth substances are of course excluded), e.g.
   a. Substances acting as a metabolic substrate.
   b. Substances inhibiting the metabolism.

These different possibilities will be tested in this chapter (sections 3, 4 and 5). We may expect that these experiments will also give some information about the processes and paths along which the change is brought about.

2. LITERATURE

As has been stated, many different opinions have been presented about the action of growth substances on the cell wall, some of which will be reviewed here. The greater part of the investigations has been confined to the plasticity of the cell wall, but the problem will be the same, irrespective whether plasticity or elasticity is considered.

HEYN (1940) is inclined to accept a direct action of growth substances on the cell wall and is in this regard at variance with his earlier statements (see below). He bases his opinion on experiments of HEYN and VAN OVERBEEK (1931) who found that the plasticity was changed by growth substances at 4° C. This might exclude an appreciable formation of wall substances, which has been proved later by BONNER (1934). In its turn this fact might imply that the synthesis of wall material is not necessary for growth and that, therefore, growth substances act directly on the cell wall. BONNER's results, however, are not quite convincing, as an effect of growth substances can only be found when the sections are treated with the growth substances for one hour at 25° C, and then removed and placed at a temperature of 2° C. As our own experiments showed (chapter 4), some change took place in the cell wall after 1 hour treatment with I.A.A. Therefore, the alleged difference between the control and the treated series may have its origin in the one hour of treatment at a high temperature. The same objection can be raised against the experiments of HEYN and VAN OVERBEEK (1931). No effect of growth substances exists when the coleoptiles, or sections, are kept at the low temperature from the beginning of the experiment. This was found in some of our own experiments (unpublished) and was also found by BONNER himself (1934). Moreover, considering the rather crude technique used by BONNER, we may expect that the formation of small amounts of wall material will not be detected, while they are sufficient to keep the primary cell wall growing. The bulk of the increase of wall material,

as found by BONNER, will be caused through the apposition of secondary layers. This process might be inhibited by low temperatures.

This hypothesis finds some confirmation in investigations about the wall structure during growth. BONNER (1936) showed that the wall structure remains the same during elongation. He suggests that the change of wall properties is brought about through a loosening of the "Haftpunkte" in the micellar network by the growth substances. This can, of course, be accomplished directly or indirectly. But BONNER is now (1936) of the different opinion that growth cannot be a simple mechanical process. To maintain the structure new material must be woven in. This is a more detailed variant of the theory suggested by SACHS (1874).

BURSTRÖM (1942) assumes that I.A.A. causes an increase of the elasticity through a direct action on the cell wall. Growth, however, would be accompanied by the formation of new cellulose micellae.

RUGE (1937) also defends a direct action of I.A.A. on the cell wall. He explains the loosening of the "Haftpunkte" as a result of the swelling of intermicellar substances under the influence of growth substances. He supports this hypothesis with experiments about the promotion of the swelling of different slime bodies by I.A.A. These experiments were performed with inadequate techniques and too high concentrations of I.A.A. BLANK and DEUEL (1943) examined the effect of I.A.A. on the swelling and the viscosity of wall substances in model experiments. In physiological concentrations they did not find an effect of I.A.A., while in higher concentrations I.A.A. showed a normal acid effect. So they rejected RUGE's hypothesis. RUGE (1942) still maintains a direct effect of growth substances on the cell wall, presuming an influence on the heteropolar bonds between 2 OH-groups of two adjacent cellulose molecules. In view of the results of many other investigators, however, a direct action seems most unlikely.

This is illustrated for example by HEYN's statement (1931) that elongation cannot be resumed after plasmolysis. This phenomenon has been found by REINHARDT (1899) for roots of different plants after plasmolysis in sucrose solutions. It can be considered as an indication for an interaction between the protoplasm and the cell wall in the process of elongation.

GESSNER (1934) found that the factors which affect the wall properties do not act when the cell has been plasmolysed. Thus the protoplasm will play an important role.

THIMANN and BONNER (1933) could not establish a direct stoichiometric relationship between the amount of growth substance entering the cell wall constituents on which it acts. They also calculated that the quantities of growth substance involved in elongation are not sufficient to form a monomolecular layer over the cell wall. These authors suggested an indirect action of growth substances on the cell wall. Their conclusions seem to be justified.

KONINGSBERGER (1947) pointed out that, assuming a direct swelling effect of growth substances on the cell wall, it is not possible to explain

the optimum curve found. In that case one would expect a sigmoid saturation curve.

SÖDING (1934, 1952) considers the intussusception of new wall material as the most important process during elongation. The wall properties are changed, but this will be an accompanying phenomenon.

In view of the results of these investigators and of the many various actions of growth substances on plants and plant cells, it does not seem likely that a direct action on the cell wall will be found. We may support FREY WYSSLING's opinion (1945, 1948, 1952) who maintained the importance of the protoplasm as an intermediary in the growth process.

3. THE INFLUENCE OF PRECEDING PLASMOLYSIS ON THE ACTION OF I.A.A.

It may be assumed that the action of I.A.A. will be affected by a preceding plasmolysis, as it is likely that at least part of the structural relation between cell wall and protoplasm will have been destroyed. To this end experiments were carried out in which sections were plasmolysed in 1 mol. mannitol. Microscopic examination showed that with this concentration of mannitol the parenchyma was completely plasmolysed. Occasionally even abnormal plasmolysis was observed. Plasmolysis could not be irrefutably established in the outer epiderm, owing to the great length of the cells, which makes it difficult to obtain intact cells. After 1 hour the mannitol was removed, the sections were rinsed thoroughly in distilled water and immersed in fresh distilled water, allowing deplasmolysis. The distilled water may contain I.A.A. so that the deplasmolysis took place in the presence of growth substance, or I.A.A. was added when the deplasmolysis had been completed. In table 18 and 19 the results are shown.

TABLE 18

The effect of preceding plasmolysis on elongation. The concentration of I.A.A. is 1 mg/l. The experimental numbers correspond to those of table 19.

| Exp. no. | % Elongation | | | | Duration of the exp. hours |
| | Plasmolysed sections | | Not plasmolysed sections | | |
	I.A.A.	Contr.	I.A.A.	Contr.	
1	25,0	14,0			24
2	17,9	5,3	45,7	6,8	18
3d	35,4	13,1			18
4a	12,8	5,8	23,0		5
b	30,2	8,0	46,9		24
5	20,3	5,4	52,9	16,7	20
6	60,5	6,4	70,4	8,9	24
7	14,5	6,5	62,9	10,5	18

Preceding plasmolysis decreases the reaction of the elongation on I.A.A. (table 18), but a reaction still exists. Elongation can be resumed after plasmolysis. The extension is also increased by I.A.A., as appears from table 19. In some experiments this increase was appreciable, in others the effect was small or absent. Exp. 3 and 4 may indicate that a longer time between the plasmolysis and the addition of I.A.A.

favours the reaction of the sections, as the extension was maximum when the I.A.A. had been added 6 hours after plasmolysis. The analysis of this phenomenon will be difficult, for the variability increases as a result of plasmolysis. Therefore a further examination is in progress, employing the growth reaction proper.

TABLE 19

The effect of preceding plasmolysis on the elastic extension. The concentration of I.A.A. is 1 mg/l

Exp. no.	I	II	III	E			
				Plasmolysed		Not plasmolysed	
				I.A.A.	Contr.	I.A.A.	Contr.
1	0	0	24	7,6	7,0	11,7	7,9
2	0	6	18	6,3	5,3	12,0	6,4
3	0	0	24	9,4	7,2		
	0	2	22	10,1	6,9		
	0	4	20	10,0	7,5		
	0	6	18	11,6	8,3		
4	0	0	5	8,7	8,0		
	0	0	24	9,3	5,7		
	0	5	19	11,9			
5	4	0	20	8,2	5,8	11,7	7,9
6	5	0	24	13,2	7,3	13,5	6,9
7	0	6	18	6,3	6,0	13,2	7,1

I. Time between cutting and plasmolysing. II. Time between plasmolysis and the addition of I.A.A. III. Time of treatment with I.A.A.

It may be concluded that plasmolysis affects the action of I.A.A. This suggests an interaction between the protoplasm and the cell wall. The unexpected result, that sections still react on growth substances after plasmolysis and deplasmolysis may be explained by the preservation of a part of the structural relation, or by a partial recovery after deplasmolysis, or both possibilities may be realized. The first possibility is supported by the close association which is generally found between proteins and the primary wall.

4. THE INFLUENCE OF SUGARS ON THE ACTION OF I.A.A

It is a well known fact that sugars promote the action of growth substances (RIETSEMA, 1950). This can be seen in fig. 13 for example. Sugars are easily used in metabolism and therefore it seemed worthwhile to examine their effect on the elastic extension. A disadvantage of the use of these carbohydrates is that it takes 3 to 5 hours before a reaction of the growth rate on sugars can be found. The starving of sections before the addition of the sugars does not shorten this lag.

The results of a number of experiments in which glucose and sucrose were tested have been compiled in table 20. Sucrose and glucose appear to increase the effect of I.A.A. Without I.A.A. the effect of sugars on the elastic extension is not so evident. This corresponds to a slight reaction of the growth rate on sugars only. The

effect of sugars is very evident when the sections are immersed in distilled water for about 24 hours before the addition of the sugars. Apparently these starved sections are short of substrate.

TABLE 20

The effect of sugars on the elastic extension 1 mg/1 I.A.A. has been used. In column 2 the time between cutting and the addition of the sugars and I.A.A. has been given.

Exp.	Petreat-ment hours	E %				Conc. sugar	Duration hours
		Contr.	Sugar	I.A.A.	I.A.A. sugar		
1	5	8,8	7,6	11,4	12,6	0,8 % glucose	20
2	5	6,6	8,1			,,	24
3	20	4,9	7,1	9,6	15,9	,,	20
4	24	6,1	7,2	11,9	12,8	,,	7
5	24	4,3	11,1	8,2	13,8	,,	28
6	24	5,1	5,9	9,7	14,4	,,	24
7	5	6,3	6,8	14,7	16,7	1 % sucrose	20
8	6	8,1	7,8	13,0	14,4	,,	19
9	5			10,2	15,4	,,	24
10	6	7,9	8,2			,,	23

In the above table the E-values are given and not the absolute value of the extension. The latter is with sugars 150 %–200 % higher than with I.A.A. alone, but part of this difference must be accounted for by the increased length attained after 24 hours. Fig. 13 (page 434), for example, shows that with I.A.A. plus sucrose the elongation amounts to more than 75 %, while I.A.A. alone gives 30 % elongation. Without any change in elastic properties the mere occurrence of such a difference of length will cause an increase in the elastic extension. As E is increased, however, the change in extension cannot be accounted for completely by the increase in length. The elastic properties must be changed.

This interaction between I.A.A. and carbohydrates indicates that the wall properties are changed through the intermediary of the protoplasm. In this relation the sugars may be used in the synthesis of new wall material.

5. THE INFLUENCE OF DINITROPHENOL ON THE ACTION OF I.A.A.

The preceding experiments suggest that the protoplasm has an active function in the change of the elastic extension by growth substances. The effect of sugars might be explained by assuming that the laying down of new material in the primary wall causes the increase of the elastic extension. In order to put this assumption to the test the effect of dinitrophenol (DNP) has been examined. BONNER (1949) and HACKETT and THIMANN (1950, 1952, 1953) have demonstrated that DNP inhibits growth and water uptake. It is shown in fig. 13 that DNP reduces the effect of both I.A.A. and I.A.A. plus sucrose on elongation. The first indication of growth inhibition is found 2–3 hours after the addition of DNP.

It had to be ascertained that DNP is not toxic to the sections. The

effect of different concentrations of DNP was therefore tested in
combination with 1mg/1 I.A.A. One of these experiments has been
presented in fig. 14. In the experiment shown in this figure 3 mg/1
DNP is already toxic, as the sections shrink after prolonged contact
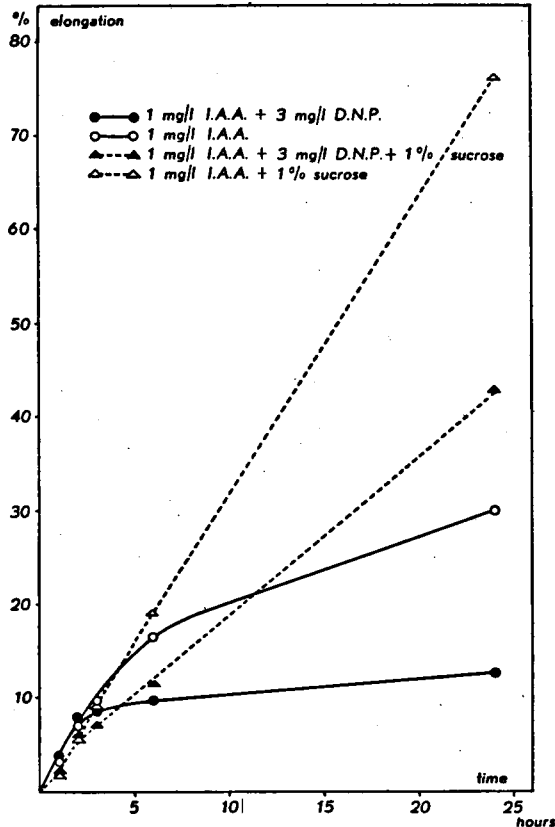with DNP and cannot be plasmolysed any more. Moreover, these

Fig. 13.   The effect of DNP on elongation.

sections were not able to start growing again when the inhibitor had
been removed. If measurements are made at the beginning of the
experiments and after 18 or 24 hours only, as is commonly done, this
phenomenon will escape observation, the more easily so, as the final
length after the shrinkage is greater than the initial length in many
cases. Conclusions based on such experiments run the risk of being
incorrect. In this regard the results of BONNER (1949) must be recon-
sidered. Identical results have been obtained in the presence of I.A.A.
plus sucrose. Lowering of the pH has the same effect as increasing
the concentration of DNP (see table 22 exp. 4).

The sensitivity of the sections to DNP differs from day to day. In the
experiment shown in fig. 14 the sections were very sensitive. Normally

3 mg/l does not show any damaging effect and often 4 mg/l can be used. A series of sections was always run for 18 to 24 hours to make sure that the DNP concentration used did not have an injurious effect.
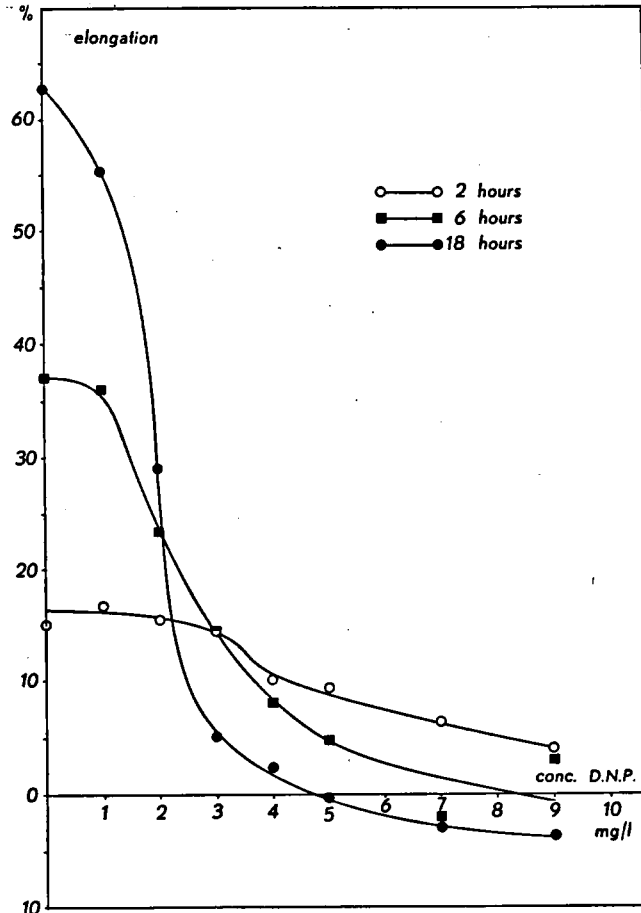

Fig. 14.   The effect of different concentrations of DNP on elongation after 2,6 and 18 hours of treatment. The sections received 1 mg/l I.A.A. together with DNP.

In a number of experiments the influence of DNP on the effect of I.A.A. and I.A.A. plus sucrose was examined. It appears from table 21 that growth is strongly inhibited and that E is decreased by DNP. The growth differences are very considerable, however, and this may complicate the interpretation. Therefore experiments were carried out in which the elastic extension and E were determined after 2 or 3 hours treatment. In this case the differences in growth are inconspicuous or do not exist at all. Differences in wall properties, if any, must therefore be brought about by the substances added.

In table 22 the results of these experiments have been collected, while exp. 6 is shown in fig. 15.

TABLE 21

The influence of DNP on the effect of I.A.A. and sucrose. Concentrations: 1 mg/1 I.A.A., 1 % sucrose and 3 mg/1 DNP

Composition of the medium	% elongation				E			
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Dist. water.	15,3	5,6		7,1	8,1	8,3		6,6
DNP	8,9	7,6			3,2	7,1		
I.A.A..	62,5	27,5	29,9	42,0	13,0	10,1	10,2	13,3
I.A.A. + DNP. . . .	39,6	18,4	12,6	16,3	8,6	7,2	6,7	6,8
I.A.A. + sucr.	105,0		76,3		14,4		15,4	
I.A.A. + sucr. + DNP	57,7		43,0		12,9		13,2	
Duration of the exp. hours	19	18	24	18	19	18	24	18

TABLE 22

The effect of DNP on the elastic extension and on E in the presence of I.A.A. Concentrations used: 1 mg/1 I.A.A. and 3 mg/1 DNP. The values given are averages of 10 sections

Exp. no.	Elastic extension			E			Duration hours
	Contr.	I.A.A.	I.A.A.-DNP	Contr.	I.A.A.	I.A.A.-DNP	
* 1	3,9	6,4	4,7	9,3	13,8	10,7	2
2	4,0	5,4	4,5	9,6	12,3	10,2	2
			3,5 *			8,5 *	
† 3		4,5	3,1		10,6	7,5	2
4a		5,5	4,7		11,9	10,2	2
† b		5,4	3,9		11,8	8,8	2
5a	3,5	4,4	4,0 §	8,2	10,3	9,2 §	2
b	2,8	5,4	4,4 §	6,7	11,8	9,4 §	3
§ 6a	3,9	5,4	4,7	9,2	12,2	10,2	2
§ b	4,2	6,0	4,9	9,8	12,7	10,6	3

  *   5 mg/1 DNP
  †   In 1/100 mol KH_2PO_4 (pH 4,5).
  §   Average value of 20 sections.

Considering these data it becomes apparent that DNP partly prevents the increase in the elastic extension, which is brought about by I.A.A. The effect of I.A.A. is decreased after 2 hours of treatment with DNP and even more after 3 hours. A low pH and a higher concentration accelerate the action of DNP (exp. 2, 3 and 4). A statistical analysis shows that the differences found are statistically significant or highly significant. For example, the chance that the very slight difference in exp. 5a originated by accident is about 7 in 1000.

The results of these experiments show that DNP intervenes with the action of I.A.A. on the cell wall. From the work of HOTCHKISS

c.s. (1944) it is known that DNP prevents the formation of high-energy phosphates and stops synthesis in the cell. This means that the change of the wall properties by I.A.A. is brought along via the synthetic activity of the cell.
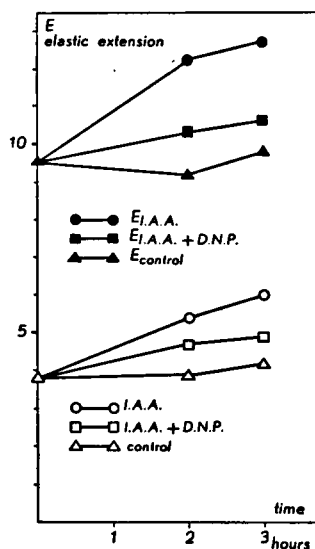

Fig. 15. The effect of 3 mg/1 DNP on the action of 1 mg/1 I.A.A.

6. DISCUSSION

The experiments described in this chapter suggest that the synthesis of wall substances is essential for the change in wall properties. The decreased effect of I.A.A. after preceding plasmolysis supports the assumption that I.A.A. acts on the cell wall through the intermediary of the protoplasm. The effect of carbohydrates points in the same direction, while the mechanism of the action is more specified by the fact that DNP affects the process. It may be tentatively concluded that growth substances stimulate directly or indirectly the synthetic activity of the protoplasm, which causes an increased synthesis of wall materials and protoplasmic constituents.

GENERAL DISCUSSION

As has been pointed out in the discussions in chapter II and III, an increase in the permeability to water or in the osmotic value of the cell contents cannot account for the higher growth rate of isolated coleoptile·sections in the presence of I.A.A. As possible causes a change in the properties of the cell wall or a stimulation of an active process of water absorption are left. KRAMER and CURRIER (1950) stated that there is as yet no sufficient direct proof for the existence of an active water absorption. The new arguments put forward by THIMANN (1951)

did not provide binding evidence either (page 392). It is impossible, moreover, to estimate the part of an active "pumping" of water in the growth process and its connection with growth substances. Attention must, therefore, be focussed on the cell wall.

It has been stated (page 419) that the physical properties of the cell wall must change in order to maintain a high growth rate for some hours. An irreversible increase in area of the wall is the most economic, as then no energy is needed to keep the absorbed water in the cells (LEVITT, 1948). Either a change in the plasticity of the wall or a stimulated active growth in area of the primary wall might be effected by growth substances, which might increase the growth rate in this way. As MÜHLETHALER (1950) found that apposition of secondary layers with fiber texture occurs in still elongating cells, the first possibility is very unlikely. This is supported further by the invariate submicroscopic structure of the primary wall during elongation. Even the fast growing antheres of grasses (FREY-WYSSLING and SCHOCH-BODMER, 1938) and the seta of *Pellia* (OVERBECK, 1934. VAN ITERSON, 1935) maintain the tube texture during elongation. Moreover, WIRTH (1946) and PRESTON and CLARK (1944) demonstrated that the amount of wall material increased during growth. The latter authors found that the amount of wall material per unit of length decreased, but this does not prove that elongation is a simple mechanical phenomenon.

The experiments reported in Chapter V of the present investigation give direct proof that the change in wall properties under influence of I.A.A. is connected with the metabolic activity of the cell. It appeared that DNP decreased the effect of I.A.A. on the elastic extension even when no growth differences could be found. This also indicates that no direct relation exists between the elastic extension and the growth rate. The difference in the time sequence of the extension and the growth rate after the addition of I.A.A. (page 426) points out the same. The change in elastic extension, however, indicates a change of the cell wall, a change which is not the consequence of elongation. The close oxygen dependency of the action of I.A.A. and the influence of preceding plasmolysis, carbohydrates and DNP on the effect of I.A.A. suggest that I.A.A. affects the cell wall through intermediary of the protoplasm.

For the mechanism of cell wall growth we may refer to MÜHLETHALER (1950) and FREY-WYSSLING (1950). MÜHLETHALER found that the individual cells of a coleoptile have inelastic centre parts and elastic poles, because secondary layers are absent at the tips of the cells only. The cells would elongate by bipolar tip growth by the interweaving of new cellulose fibrils. This conception is a modern variant of PFEFFER's "aktives Intussuszeptionswachstum" (1893). This hypothesis accounts for the high energy expenditure during elongation, as new fibrils must be synthesized.

The results of the present investigation may be explained by assuming that I.A.A. stimulates the biosynthesis of new fibrils and so the interweaving of these fibrils in the primary wall. MÜHLETHALER found

cells with long thin-walled poles in growing coleoptiles. In the presence of I.A.A. these poles might cover a larger area, for instance because the apposition of secondary layers temporarily lags behind. This will result in an increase in the elastic extension, as the elasticity is reduced by the secondary layers with fibre texture. The decrease in extension when the elongation slows down (fig. 10, page 423) might be explained by a relatively more pronounced apposition of secondary layers.

Comparable observations were made by EKDAHL (1953), who found that the extension of mature hair-cells of the root was about 1,5 %, while growing hair-cells were extended up to 11,2 %. In the root hairs the elastic extension decreased with the increase in length of the hairs. The growing apical wall was very extensible, but only within the very tip region less than 25 mikron long. EKDAHL also observed apposition of secondary layers immediately below the tip.

If the hypothesis outlined above is correct, the magnitude E (page 422) has little significance, as the completed parts of the wall attribute but a little to the elastic extension. In that case the extension is not directly proportional to the length of the cell. The effect of I.A.A. will then be much greater than appears from the E-values and will nearly account for the total absolute increase in the extension.

The plasticity of the newly formed parts of the wall may also be expected to be higher than that of the mature parts. This offers an explanation for the increase in plasticity after I.A.A. treatment found by HEYN (1931) and SÖDING (1934). The increase both in plasticity and in elasticity, however, will be secondary phenomena. These considerations are based on morphological studies of the cellulose skeletons of primary walls. What part the pectic substances play in the growth of the cell wall is still obscure. Some authors consider the pectic substances as the most essential part of the wall during growth (KERR, 1951. EKDAHL, 1953), but this assumption is still wholly unproven.

We do not know what metabolic processes are affected by growth substances and how the processes are linked together, no adequate techniques are available to study cell wall growth in vivo and we can only guess at the mechanism of the action of growth substances. It may be tentatively suggested that growth substances act on lipophilic phases or membranes within the protoplasm (ANKER, 1953). In the present investigation I.A.A. appeared to have a modifying effect on the ectoplasm. Within the protoplasm chondriosomes, for example, may be coacervates or may at least be separated from the remaining protoplasm by a complex-coacervate film. Such inner membranes may be affected by growth substances.

The final conclusion of the present paper is that elongation is an active growing of the cell wall by intussusception, which is affected by growth substances, presumably by a physico-chemical stimulation of one or more of the enzymatic processes leading to the biosynthesis of new cell wall materials. The synthesis of protoplasmic constituents during elongation (FREY-WYSSLING, 1945) may be stimulated in the same way. No need of an active (non-osmotic) water absorption

exists, for the osmotic value of the cell contents and the active growth of the cell wall will be able to maintain a suction force (page 000). Recently LEVITT (1953) [1] and BURSTRÖM (1953) [2] also did away with an active water absorption and with its possible function in the process of elongation.

SUMMARY

A study was made of the effect of indole-3-acetic acid (I.A.A.) on growth and water intake of isolated *Avena* coleoptile sections in distilled water. Both processes were greatly promoted by I.A.A. Attention was focussed on the mechanism of the action of I.A.A.

I.A.A. (1 mg/1) showed a tendency to decrease the permeability to heavy water, while a low pH increased this magnitude. The effect of I.A.A. and pH on growth and water intake could not be explained by their effect on the water permeability.

The sections always maintained a positive suction force, even after prolonged immersion in I.A.A. solutions or in distilled water. This suction force, which is found after prolonged immersion, originated from the active growth of the sections and was significantly increased after short times of treatment with I.A.A.

The osmotic value of the cell contents was not increased by I.A.A., but it decreased in proportion to the increase in length of the sections. I.A.A. did not stimulate the formation of osmotically active substances.

The elastic extension of the sections was increased by I.A.A. This indicated a change of the cell wall. It was demonstrated that the greatest increase in elastic extension occurred during the first two hours after the addition of I.A.A. No direct relation could be established between the elastic extension and the growth rate.

A further analysis showed that preceding plasmolysis decreased the effect of I.A.A. on the extension. Sucrose and glucose increased the effect of I.A.A., which increase might be partly due to a possible increase in the osmotic value by sugars. Dinitrophenol inhibited the action of I.A.A. on extension and on growth. These results indicated that I.A.A. changed the cell wall through intermediary of the active metabolism of the protoplasm.

It is suggested that elongation is an active growth in area of the cell wall by intussusception. I.A.A. is assumed to promote the bio-synthesis of wall substances by a physico-chemical stimulation of one or more enzyme systems. This hypothesis accounts both for the high energy consumption and for the change in wall properties after the addition of growth substances to coleoptile sections.

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