Botany. — *On the influence of pH on the growth of Avena coleoptile sections.* (Preliminary note.) By J. RIETSEMA. (Communicated by Prof. V. J. KONINGSBERGER.)

(Communicated at the meeting of September 24, 1949.)

Introduction.

STRUGGER (1932, 1933) was the first to investigate the influence of pH on plant growth in connection with the action of growth substances. He found that the growth of roots and hypocotyls of *Helianthus* in Na-acetate buffers showed two pH-optima, namely at pH 4.25 and 5.5, whereas at pH 5.1 growth was minimal. The relation of this phenomenon to growth substances was supposed to be an indirect one.

J. BONNER (1934) failed to reproduce two pH optima for "acid-curvatures" of *Avena* coleoptiles. According to this author, the curve obtained by plotting the "acid-curvatures" against the internal pH of the coleoptiles closely equals the dissociation curve of the growth promoting substance produced by *Rhizopus suinus* (DOLK and THIMANN (1932)). The internal pH of the cells was supposed to be changed by the external solution.

A. M. A. VAN SANTEN (1938, 1940) confirmed this fact in studying the straight growth of small sections of *Avena* coleoptiles and of roots of *Pisum sativum* and *Helianthus annuus*; the "growth-external pH" curves showed a close similarity to the dissociation curve of both auxin-a and indole-3-acetic acid (I.A.A.). Moreover Miss VAN SANTEN (1940) stated, that STRUGGERS results are disputable, since the condition of the plants used was unsatisfactory and Na-acetate buffers are toxic to roots of *Helianthus*. SAKAMURA and KANAMORI (1935) showed the toxicity of Na-acetate buffers to root hairs of *Brassica*.

The pH of the cell contents was assumed to be changed by the external solution and to obtain the value of the latter gradually, whereas growth is exactly proportional to the concentration of undissociated molecules of the growth hormone present inside the cell.

D. M. BONNER (1938) revealed the fact that several synthetic growth substances are equally active in the pea test, with regard to the concentration of undissociated molecules only. Also it was stated, that the internal pH of the tissue was changed by the external buffer solution. This internal pH was measured with a glass electrode after crushing the tissue. By plotting these pH values against the curvatures a graph was obtained showing a close correlation with the dissociation curves of the growth substance used. On the other hand KÖGL, HAAGEN SMIT and ERXLEBEN (1933) discovered that the activity of solutions of auxin-a in the *Avena* test is improved by the addition of small amounts of KCl and acetic acid. It was shown by DOLK and THIMANN (1932), that indole-acetic acid, produced by

Rhizopus suinus enters the cell in the undissociated state only. ALBAUM, KAISER and NESTLER (1937) proved this to be true for the diffusion of indole-3-acetic acid molecules into the vacuole of *Nitella* cells. It may be dangerous, however, to apply these results to epidermal cells of a coleoptile, since *Nitella* consists of threads of single cells, whilst the coleoptile is a real tissue.

In 1938 THIMANN and SCHNEIDER succeeded in demonstrating the same phenomenon in experiments concerning the accelerating action of I.A.A. on protoplasmic streaming in epidermal cells of *Avena* coleoptiles.

All these experiments were of short duration. In prolonged experiments (24 hours) Miss VAN SANTEN (1938) obtained the same results. However, no full report of the results was given and the figures presented are unsufficient to substantiate any conclusion.

Summarizing this brief survey of literature we may state, that two different actions of a buffer solution have been taken into account:

I. Action within the cell: Growth is determined by the external pH, which changes the pH of the cell contents. Only the undissociated molecules of the total amount of growth substance present inside the cell are promoting growth.

II. Action outside the cell: By adding growth substance to the external solution growth is promoted only by those molecules diffusing into the cells. The number of these molecules is determined by the external pH, for only undissociated molecules are able to penetrate into the cells.

According to VAN SANTEN (1940) both actions may cooperate, but in *Avena* coleoptiles not in the sense of a mere addition.

HITCHCOCK and ZIMMERMAN (1938) raised serious objections against these views. They claim to have shown that the effect of buffer solutions on the root formation in leaf cuttings of tomato plants merely depends on the molarity of the buffer solution used and not on its pH. High activity was obtained always at complete dissociation of the growth substance. And, since the assumption that the internal pH can be lowered by external buffer solutions, never has been proved, it is concluded that the dissociation hypothesis has neither application to root formation in tomato leaf-cuttings, nor to other material.

POHL (1948), refuting the dissociation hypothesis, supposed both an increase of the hydrogen ion concentration and addition of indole-3-acetic acid to raise the growth of *Avena* coleoptile sections by increasing the water permeability.

It is evident that the problem of the pH effect on growth needs further investigation. First we present some experiments dealing with the effect of external buffer solutions on the pH of the cell contents; second some results concerning the influence of the external pH on the growth promoting activity of some growth substances are given.

II. *Material and methods.*

The "Victory oat" strain from Svalöf was used as a test organism. Three days old coleoptiles grown in complete darkness in an air-conditioned room at 23° C and 96 % relative humidity were cut into small sections of $1\frac{1}{2}$ mm, 5—$6\frac{1}{2}$ mm from the tip, with a microtome after VAN DER WEY (1932). For each pH value at least one series consisting of 20 sections was used per experiment.

According to VAN SANTEN (1938) the sections were mounted on a slide by means of a narrow strip of pure vaseline. The slides were placed into a petri dish with 100 ml buffer solution. The length of the sections was measured under a microscope at low power magnification in orange light. Before and after measuring the sections remained in complete darkness. During the experiment the solution was aerated intensely. In this way well reproducible results were obtained.

The buffer solutions were prepared of mixtures of 0.01 m KH_2PO_4 and 0.01 m K_2HPO_4. As VAN SANTEN showed a toxicity of Na-ions for roots only potassium salts were used to avoid any deleterious action of Na. The pH measurements were performed with a quinhydrone electrode.

The synthetic growth substances used were an indole-3-acetic acid preparation from KAHLBAUM, Berlin, and one from the Amsterdamsche Chinine Fabriek. Both indole-3-acetic acid samples had the same acivity pro mg.

pH measurements of the cell contents were performed by means of a micromanipulator after DE FONBRUNE (1937). After immersion during 24 hours in a buffer solution a section of a coleoptile was washed twice with distilled water and was mounted on a slide in such a way, that it could not be pushed aside by the micropipet while piercing the cell wall. Immediately after adjusting the section a small drop of indicator solution (prepared after J. SMALL (1928)) was injected into the cell and the colour was observed. The two main colours of the indicator on the acid and on the alkaline side were taken into account only. Intermediate colours were not taken into consideration (SMALL's "Range Indicator Method"). By using various indicators it became possible to estimate the pH between two limits. Table I (taken from J. SMALL (1928), chapter 8) shows the indicators used and the pH limits for the principal colours.

III. *Experimental.*

A. pH of the cell contents.

In order to determine the pH of the cell contents several methods have been used by various investigators. Only a few of them applied the direct method of injecting indicator solutions into the cells.

J. BONNER (1934) and D. M. BONNER (1938) crushed the tissue and measured the pH of this material after adding som distilled water.

The following considerations may show that this method is not reliable:

a. Crushed tissue consists of a mixture of cell walls, disorganized pro-

TABLE I. Characteristics of the indicators used.

Indicator	Alkaline Colour Range	pH	Acid Colour Range	pH
Bromo-cresol purple	pale blue to deep purple	6.2	yellow	5.9
Methyl red	yellow	5.6	pale pink to deep red	5.2
Benzene-azo-a-naphthylamine	yellow	4.8	pale pink to deep red	4.4
Bromo-cresol green	pale green to deep blue	4.4	yellow	4.0

toplasm and vacuole contents. Since these constituents in a living cell each may have a different pH, the significance of the pH determinations of crushed tissue is doubtful. *b.* After crushing most of the CO_2 formed by the cell metabolism will disappear since in a living cell the CO_2 pressure markedly exceeds that of the open air. As CO_2 lowers the pH, crushing causes a pH increase of the cell contents (SMALL, 1928). *c.* Disorganizing protoplasm often results in a sudden decrease of the pH, as has been observed by CHAMBERS (1932).

The *Nitella* cells, ALBAUM et al. (1937) worked with, are more favorable material. The vacuole contents of a number of cells were collected and the pH was determined by means of a glass electrode. As to this procedure only the objection under *b* remains. No pH changes of the vacuole contents were observed, even if the cells remained in a buffer solution during one hour. But as to the pH of the protoplasm no information was obtained.

The above mentioned objections are avoided by determining the pH by means of vital staining, but unhappily most indicators are no vital dyes. Consequently they do not penetrate into the living cell (GUILLIERMOND, 1941).

A more exact method consists of microinjection of indicator solutions, as described by REISS (1926), SMALL (1928), PLOWE (1931), CHAMBERS and CAMERON (1932).

The present experiments showed that the pH of the cell contents remains constant throughout a period of 24 hours in a 0.01 m phosphate buffer solution. Though it was difficult to prevent a disorganization of the protoplasm, followed by typical changes shown in the cell structure after killing caused by too much stirring of the micropipet, we succeeded in a number of cases in observing the indicator within the cytoplasm. So it proved to be possible to estimate the pH of the vacuole and the cytoplasm separately. Table II summarizes the results of several experiments.

It must be emphasized however that these results are preliminary; the experiments will be continued and extended.

As can be clearly seen from table II the pH of a living cell remains constant during a period of 24 hours. This holds even for tissue in a toxic

TABLE II. Influence of the pH of the medium on the pH within the cell.

External pH	Indicator	Cytoplasm		Vacuole	
		Colour	pH	Colour	pH
4.2	Bromo-cresol purple	yellow	<5.9	yellow	<5.9
	Methyl red	yellow	>5.6	yellow	>5.6
5.8	Bromo-cresol purple	yellow	<5.9	yellow	<5.9
	Methyl red	yellow	>5.6	yellow	>5.6
8.3	Bromo-cresol purple	yellow	<5.9	indiff.*)	5.9-6.2
	Methyl red	yellow	>5.6	yellow	>5.6
3.4**)	Methyl red	indiff *)	5.2-5.6	red	<5.2
	Benzene-azo-a-napht. am.	yellow	>4.8	indiff.*)	4.4-4.8
	Bromo-cresol green	blue	>4.4	blue-green	>4.4

*) Indifferent means, that the colour was vague and intermediate between the two principal colours.

**) The coleoptile sections had lost their turgescence and may be considered dead.

buffer solution of pH 3.4 in which both cytoplasm and vacuole showed a considerably higher pH than the surrounding solution. In a living cell both cytoplasm and vacuole show pH values between 5.6 and 5.9.

These results are to be expected from the reviews on this subject by SMALL (1928, Chapters 16 and 17).

The constancy of the pH of the vacuole can be understood from considerations by ARISZ (1945). This author concluded from theoretical arguments as well as from dates derived from both literature and own experiments that, whilst the protoplasm of a cell is permeable to inorganic ions and aminoacids, the permeability of the tonoplast is different.

Now, the pH of the vacuole seems to be of minor importance for growth in contrast to the pH of the protoplasm. But even the pH of the protoplasm proved to remain constant. We must take into consideration however that the pH measurements of the protoplasm only bear upon the inner layers. The method used gives no information on the outer layers.

The mere fact however, that the hydrogen-ion concentration influences growth, means that in the protoplasm changes in the structure must have taken place. These changes most probably have been caused by a new ion-equilibrium between the buffer solution and the protoplasm (ARISZ, 1945).

Not yet published experiments on protoplasmic streaming give us further information on the influence of buffer solutions on the inner protoplasmic layers. They have shown that the rate of protoplasmic streaming in

epidermal cells of a coleoptile remains fairly constant after a 24 hours stay in a 0.01 m phosphate buffer of various pH. It seems highly improbable that changes in the ionic composition of the protoplasm are not accompanied by changes in the rate of protoplasmic streaming. Vid. STRUGGER (1926, 1928), COLLA (1929), SEIFRITZ (1943, p. 78).

If the dissociation theory with regard to the natural growth substance present inside the cell is true, this implies that the reaction of the medium as concerned with growth is limited to the outer layers.

In this respect WENT's assumptions (1938) that the action of indole-

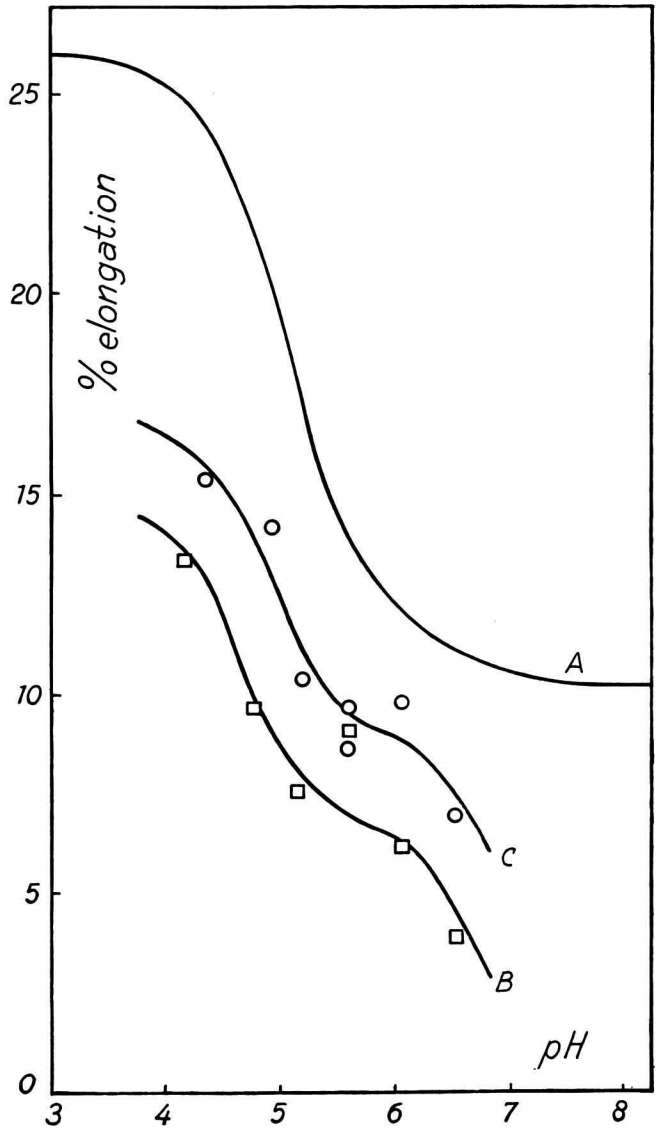

Fig. 1. Relation between the growth of *Avena* coleoptile sections and the pH of the medium. Curve *A* after VAN SANTEN (1938). Curves *B* and *C* original.

3-acetic acid on protoplasmic streaming takes place at the surface of the protoplasm may be of importance. The effect of the natural auxin on actual growth may proceed there as well.

B. Experiments on growth.

§ 1. *The influence of the hydrogen ion concentration.*

In accordance with Miss VAN SANTEN's results (1938, 1940) growth after 24 hours proved to be optimal at pH 4.2 and to decrease towards higher pH values. Compared with her experiments the growth rate is lower and more variable. This is illustrated by fig. 1. Curve *A* represents the relation between growth and pH after VAN SANTEN, whereas curves *B* and *C* are representing two out of our concordant experiments. Moreover, from pH 6.5 growth decreases more rapidly. The cause of the greater variability still remains obscure.

§ 2. *The influence of pH and glucose.*

Addition of glucose to the medium increases growth to a considerable extent, independent of the pH of the surrounding solution, as is shown in fig. 2 and table III.

TABLE III. Influence of pH and glucose on the growth of *Avena* coleoptile sections.

pH	% Elongation after 24 hours		Difference
	control	½ % glucose	
4.1	13.3 ± 0.8	21.7 ± 1.0	8.4 ± 1.2
4.8	9.7 ± 0.4	20.6 ± 0.6	10.9 ± 0.7
5.1	7.6 ± 0.5	16.4 ± 0.7	8.8 ± 0.7
5.6	9.1 ± 0.4	20.1 ± 0.9	11.0 ± 1.0
6.1	6.2 ± 0.5	15.0 ± 0.7	8.8 ± 0.9
6.5	3.9 ± 0.4	12.6 ± 0.9	8.7 ± 1.0

Consequently two processes are involved in growth: a "glucose process" independent of pH and, moreover, a pH sensitive process. The effect of both processes accumulate.

Experiments on the relation between growth and the glucose concentration have made clear that ½ % glucose is suboptimal. Hence in our experiments the glucose concentration is the factor limiting growth. Since the medium does not change the effect of glucose, we may conclude for the present that neither the permeation of glucose into the cells, nor the process in which it is involved, is influenced by the surrounding solution.

§ 3. *The influence of pH and growth substance.*

It was stated in the introduction that the experiments by VAN SANTEN (1938) on the influence of the hydrogen ion concentration on the action of indole-3-acetic acid need extension to conclude to an effect of the pH with certainty. She showed that the growth rate was affected in buffer solutions

to which indole-3-acetic acid had been added in a concentration of 1 mg/l at pH 4.18 and pH 7.0 and stated that the effect of other pH values corresponds. This means probably, that the growth-pH curve after adding heteroauxin coincides with the dissociation curve of the growth substance. Conformably in the more acid buffer solutions growth was more pronounced. 1 mg/l indole-3-acetic acid caused an increase of about 14 %

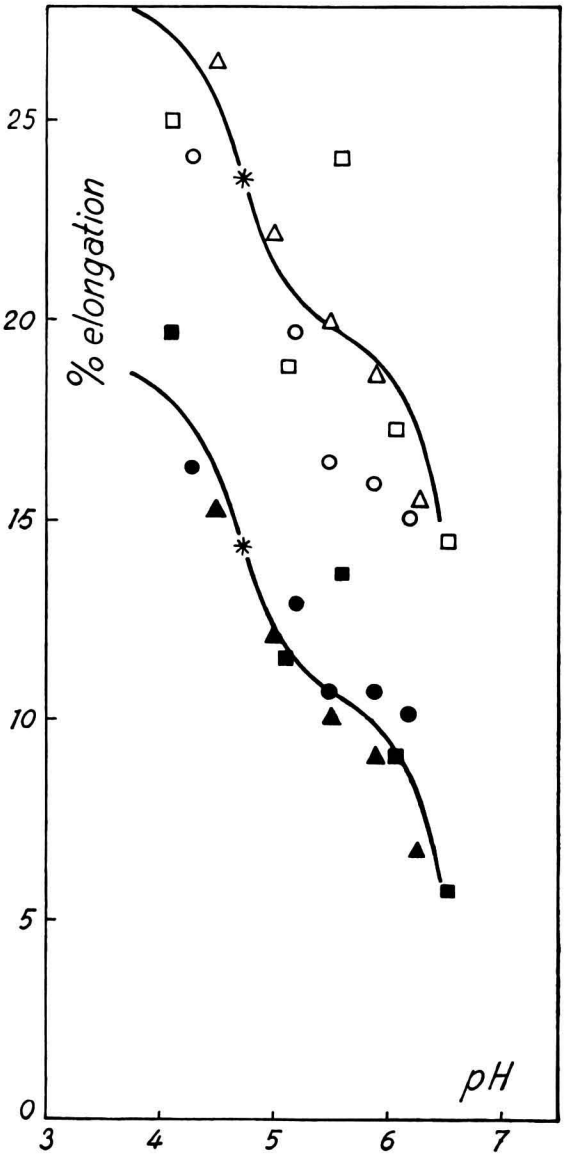

Fig. 2. The influence of pH and ½ % glucose on the growth of *Avena* coleoptile sections. The lower curve refers to growth without glucose, the upper one refers to growth with glucose, both after 24 hours. For the comparability the average values at pH 4.70—4.75 were used as reference points (indicated by asterisks) to calculate the other values.

elongation. In 1940 VAN SANTEN explained the action of the hydrogen ion concentration in terms of the penetration of the undissociated growth substance molecules into the protoplasm. As Miss VAN SANTEN's experiments have not been published in extenso they were repeated on a larger scale.

A concentration of 0.2 mg/l was used instead of 1 mg/l. As the latter concentration is optimal, salt solutions in a certain composition of the medium might permit the I.A.A. molecules to enter the protoplasm to such a degree, that also inside the cell the optimal I.A.A. concentration is reached, whilst in other media this might not be the case. If so, the growth rate at various pH cannot be compared.

The growth was measured 3, 8 and 24 hours after starting the experiment. After the first interval growth proved to be optimal at pH 4.2. When time passed on, this optimum shifted slightly to the alkaline side; after 8 and 24 hours it was moved to pH 4.5 and 4.8 respectively. At the latter moment an extremely low growth rate was observed. At prolongation of the experiment the shape of the curve remained constant. VAN SANTEN

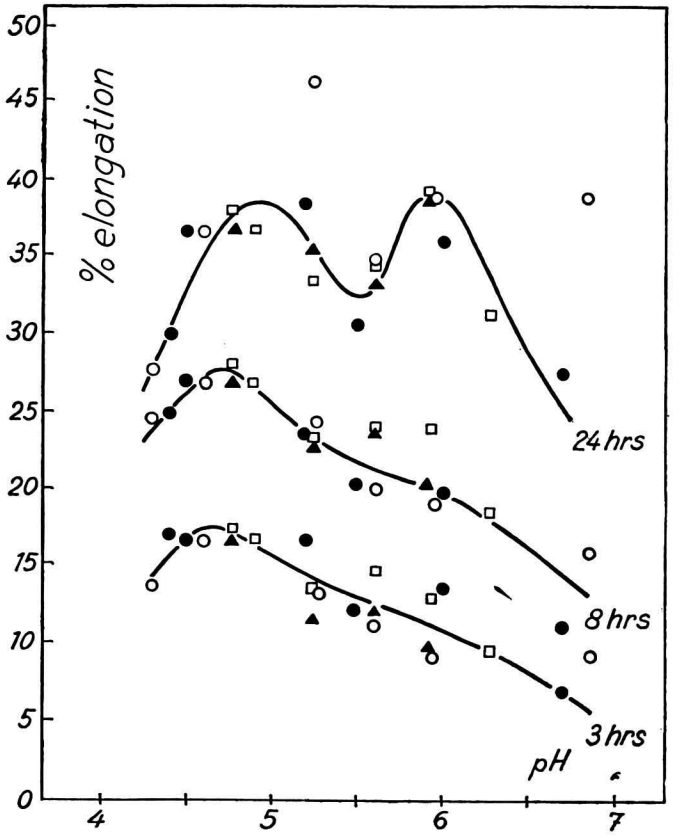

Fig. 3. Relation between the growth of *Avena* coleoptile sections and the external pH after the addition of 0.2 mg/l indole-3-acetic acid. Growth is recorded after 3, 8 and 24 hours in % elongation. For the comparability the average values at pH 4.6—4.9 were taken as reference points to calculate the other values.

(1940) also observed a shift of the optimal pH for growth of roots of *Pisum sativum* to a less acid range. May be the action of the added growth substances is more sensitive to acids than the autonomous growth.

Moreover a remarkable phenomenon was observed. At 10—12 hours from the beginning of the experiment growth between pH 5.6 and 6.2 increased, resulting in a second optimum. At both lower and higher pH values a retardation proved to occur. These results are demonstrated by fig. 3.

It is obvious that these results are in contradiction to the dissociation theory of growth substances.

POHL (1948) did not observe the second optimum, for his experiments were not extended over a 24 hour period but lasted for only 6 hours.

In this respect it is worth mentioning that heteroauxin, though a natural occurring substance, has not definitely been proved to occur in the *Avena* coleoptile. It is generally accepted, that the normal growth substance of these organs consists of auxin-a. Since the latter substance is not available in a pure cristalline state, it is not possible to compare its action with indole-3-acetic acid.

Recently WILDMAN and BONNER (1948) claim to have shown, that the bulk of the growth substance in the *Avena* coleoptile consists of indole-3-acetic acid. In a future paper some experiments will be published, to show that in the *Avena* coleoptile, besides an alkaline stable growth substance, an acid stable growth substance occurs as well. It is highly probable that this substance is identical with auxin-a.

It would be of great importance to compare the action of auxin-a and indole-3-acetic acid, but until we cannot dispose of pure auxin-a, an exact comparison is not possible.

§ 4. *Interaction of pH, growth substance and glucose.*

In the preceding paragraphs experiments were described, carried out with glucose and indole-3-acetic acid added separately. It was shown that both substances promote growth, but glucose acts independent of the pH, whilst the action of indole-3-acetic acid is pH sensitive. From these results it was concluded, that the action of both substances is of a different nature.

We next investigated, whether an interaction of both substances occurs after adding 0.2 mg/l indole-3-acetic acid and ½ % glucose simultaneously. Fig. 4 shows the results: two pH optima arise, both at the same values as those observed with hetero-auxin only (fig. 3).

Nevertheless there are some differences. The growth influenced by I.A.A. at pH 4.8—5.2 and at pH 5.9 is equal, but after the addition of glucose the growth at pH 5.9 is far more pronounced, numbering on an average 78 % at pH 5.9 and 66 % at pH 4.8.

Another difference from the results of § 2 and § 3 is the elongation caused by both substances seperately. At pH 4.8 ½ % glucose causes about 10 % elongation, growth with indole-3-acetic acid amounts to 37 % and growth with both substances together amounts to 66 %.

Hence the influence of glucose on growth is stimulated by the addition of heteroauxin, viz. 10 % and 29 % resp.

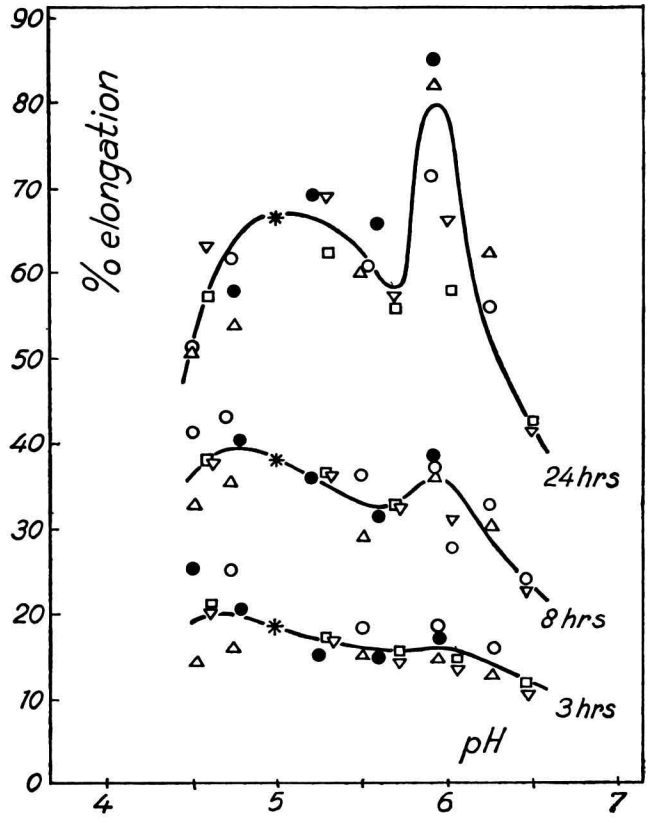

Fig. 4. Relation between the growth of *Avena* coleoptile sections and the external pH after the addition of 0.2 mg/l indole-3-acetic acid and ½ % glucose. Growth is recorded after 3, 8 and 24 hours in % elongation. For the comparability the average values at pH 5.0 were taken as reference points (indicated by asterisks) to calculate the other values.

We may conclude from these experiments that glucose interacts with indole-3-acetic acid, the effect dominating at pH 5.9, but well defined at other pH values as well. The action of glucose may be dual, as it promotes growth also without I.A.A.

COMMONER and THIMANN (1941) supposed, that indole-3-acetic acid exerted an effect on the respiration mechanism. It cannot be decided yet, whether this action holds true and may be the cause of the observed phenomena.

V. *Summary.*

1. From experiments on the pH of the cell contents it is concluded, that only the pH of the outer layers of the protoplasm may be influenced by the medium. The pH of both the inner layers and the vacuole is not to be influenced in this way, although some effect of the salt solutions on the

inner layers may not be excluded. Probably a new ion equilibrium between the medium and the protoplasm is the cause of the pH effect.

2. Experiments on the growth of *Avena* coleoptile sections confirmed the results of VAN SANTEN (1940) with regard to the effect of the pH on growth. Optimal growth occurs at pH 4.2.

3. Glucose added to the medium causes a considerable growth promotion; the influence of the pH on growth is unaffected. Obviously two processes are involved in growth.

4. Indole-3-acetic acid promotes growth dependent of the pH. Optimal growth occurs at pH 4.8—5.2 instead of pH 4.2. Apart from this optimum a second one is observed between pH 5.6 and pH 6.0, caused by an increase of the growth rate after 10—12 hours in this pH range.

5. The combined action of heteroauxin and glucose also results in two pH optima, the optimum at pH 5.9 being far more pronounced than without glucose. At other pH values a stimulation of the growth also is observed. These results indicate that the two substances interfere.

Acknowledgements.

These investigations were carried out in the Botanical Laboratory of the State University, Utrecht, and were made possible by a grant from the Stichting voor Zuiver Wetenschappelijk Onderzoek in Nederland. The author owes much to Dr. H. P. BOTTELIER and to Dr. J. B. THOMAS for their valuable criticism and aid and to Miss J. COOPS for her assistance with the experiments.

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