

THE INFLUENCE OF THE pH ON THE GROWTH
AND THE GEOTROPISM OF DECAPITATED AVENA
COLEOPTILES SUPPLIED EITHER
WITH INDOLEACETIC ACID OR WITH
INDOLEACETONITRILE

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INTRODUCTION

If the growth of a tissue when it is supplied from the outside with an acid auxin, proves to be either promoted or inhibited according to the pH of the medium, these effects might be due to a different degree of dissociation of the auxin, as the latter might influence the rate of its penetration into the cytoplasm.

The pH of the surrounding medium, however, may change in addition the acidity of the cytoplasm, and thus influence the activity of the enzymes which determine the rate of cell elongation.

As to the latter possibility, in earlier publications a complete independence of the internal pH of the external one has been defended by ALBAUM et al. (1937) and by RIETSEMA (1950), whereas a complete adjustment has been claimed by VAN SANTEN (1940). In more recent papers, however, the question of an adjustment of the internal pH is discussed with great caution (SIMON and BEEVERS, 1952; BLACKMAN and ROBERTSON-CUNINGHAME, 1953). Probably one has to distinguish between a pH occurring in the greater part of the cytoplasm and the various H-ion concentrations prevailing in the different micro-environments that must be present inside the cytoplasm. The latter are possibly of primary importance for the auxin *action*, while the pH prevailing in the greater part of the cytoplasm might be essential for the auxin *transport*, e.g. in a process like the geotropic curvature, which is studied in the present paper. If the auxin transport in the lateral direction would be accomplished by the "geo-electric effect of BRAUNER", it would considerably be facilitated by the presence of auxin in ion-form.

A complete adjustment of the internal pH to that of the medium, on the other hand, would involve that, for instance at pH = 4, no more than 10 % of the indoleacetic acid inside the cytoplasm would be dissociated. This decrease in the amount of available anions could become a limiting factor in the geotropic reaction if this process really is caused by an unequal distribution of auxin by means of electric forces.

By using both a dissociable (IAA) and an undissociable auxin (indoleacetonitrile, abbrev. IAN) in the present investigation, it was

tried to discriminate between the effect exercised by the pH of the medium on the dissociation of IAA and its possible effect on the pH of the cytoplasm.

A somewhat similar trend of thought was at the base of the experiments of AUDUS (1949) in which he studied the influence of the pH on root growth, and compared its inhibition by 2,4-D and by the non-acid inhibitor coumarin. With the latter substance root growth inhibition appeared to be independent of the pH.

An analogous result was obtained in our own experiments, for with the non-acid IAN no effect of the pH was found either on the growth of the coleoptiles or on their geotropism. With *limited* amounts of IAA, however, both processes were markedly influenced. With *abundant* IAA, on the contrary, no effect of the pH was visible. The results suggest that the influence of the pH must have been due entirely to its effect on the rate at which IAA penetrates into the cytoplasm.

METHODS

A detailed description of the method has been given earlier (ANKER, 1954, 1956). Decapitated *Avena* coleoptiles (1 mm removed) were kept horizontally or vertically for the experimental period of 75 minutes in aerated solutions of the required composition. Due to the aeration the solution was constantly stirred, which is advisable in experiments on the influence of the pH on a submerged tissue (AUDUS, 1949).

The solutions were made up from tap water, KH_2PO_4 (0.01 M), varying amounts of H_3PO_4 and either IAA or IAN. The pH, which did not change during the experiments, which lasted only 75 minutes, was measured potentiometrically.

The air humidity in the room in which the experiments were made varied from 90–96 %. The temperature was kept constant at 23° C. Only phototropically inactive orange light was admitted throughout the cultivation of the test material as well as during the experiments.

The curvatures or the elongation of the coleoptiles were measured on shadowgraphs taken at the close of the experiment. The standard deviation of the mean curvature was about 1°. When the curvatures were small, due to a supra-optimal auxin-concentration, the variation was larger and the standard deviation sometimes exceeded 2°. The measurements of the elongation always showed a great uniformity. The standard deviation of the mean increase often remained in the vicinity of 5 % and seldom reached 10 %. Each experiment was at least twice repeated. In each of the Figures 1–4 the results of one typical experiment are shown.

RESULTS

Lowering the pH inhibits geotropic curvature in experiments with IAA

In previous experiments (ANKER, 1954, 1956) it was found that a 0.075–0.1 mg/l concentration of IAA is about optimal for the regulation of the geotropic reaction of decapitated coleoptiles at

pH = 7.8. If, however, as was done in the present experiments, the acidity of the medium is increased to a pH < 6.1, then the geotropic curvature rapidly decreases at the above-mentioned IAA concentration. This is shown in Fig. 1.

At pH-values > 7, IAA is completely dissociated, whereas the percentage of non-dissociated IAA rapidly increases in media with a pH below 6 (see Fig. 1). It is often assumed that the auxin molecule

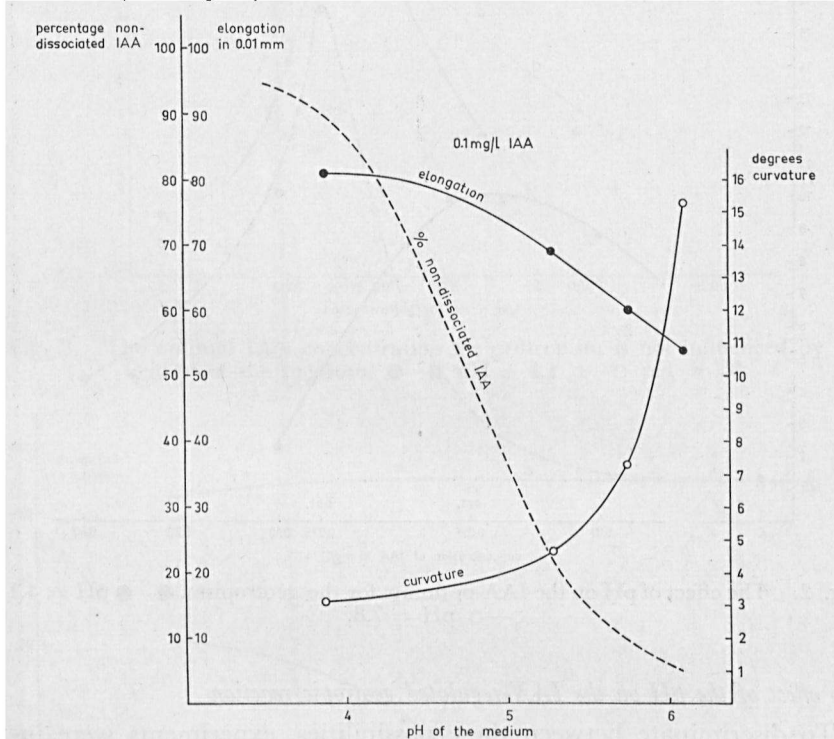


Fig. 1. The effect of pH on elongation and geotropism; 1 mm decapitation; 0.1 mg/l IAA.

penetrates easier into the cytoplasm than the anion does. Since the geotropic reaction is confined to a narrow range of auxin concentrations (ANKER, 1956), the observed inhibition might be due to a rise of the internal IAA-level above the optimum value for the geotropic curvature. This supposition was confirmed by the results of the following experiments.

Effect of the pH on the IAA-optimum for the geotropic reaction

In these experiments the relation between curvature and concentration was investigated at pH = 4.1 and at pH = 7.8. Although the shapes of the curves (Fig. 2) from which the relation is seen, show some difference, it is evident that at pH = 4.1, the concentration needed for maximal response was only one third of that required at pH = 7.8.

A shift of the optimal IAA concentration to a lower value, however, may theoretically be due (1) to an actual increase of the amount of IAA that penetrated into the cytoplasm (as suggested above), and (2) to an increase of the sensitivity of the cytoplasm to auxin, or to both these causes.

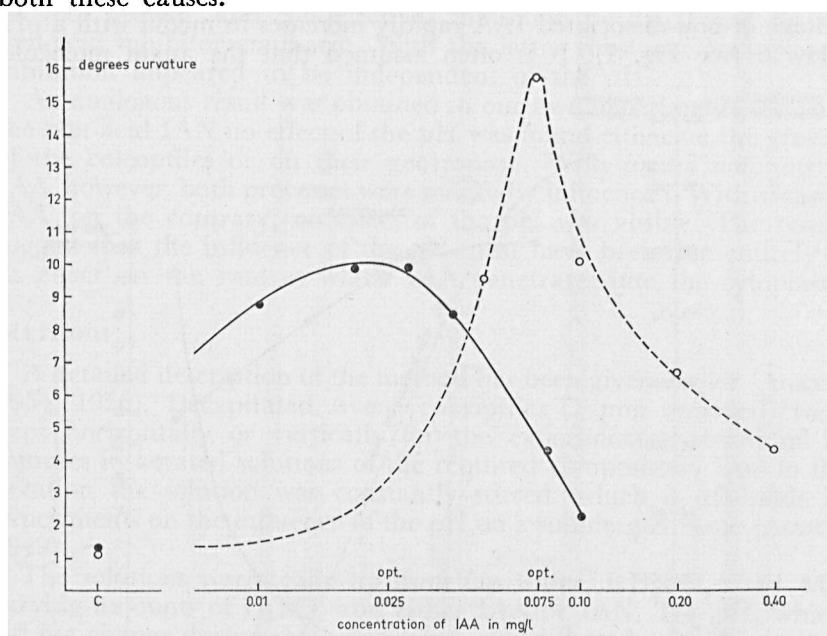


Fig. 2. The effect of pH on the IAA-optimum for the geotropism. ●—● pH = 4.1
○—○ pH = 7.8.

No effect of the pH on the IAN-regulated geotropic reaction

To discriminate between these possibilities, experiments were included with indoleacetonitrile. As the auxin does not dissociate, the pH of the medium does not affect the growth substance outside the cell. Fig. 3 shows that when IAN was added in a range of concentrations, at pH = 4.1 as well as at pH = 7.8, the maximal geotropic curvature occurred at the same IAN concentration (0.0075 mg/l).

This result strongly suggests that neither the penetration of IAN into the cytoplasm nor the sensitivity of the cytoplasm to IAN is changed by lowering the pH of the medium from 7.8 to 4.1.

Effect of the pH on straight growth

As a further test of the hypothesis, that the effect of lowering the pH on the geotropism of the coleoptiles in the experiment with IAA is due to a facilitation of its penetration into the cytoplasm, the way in which the rate of *straight growth* depends on the acidity of the medium, was investigated. If our hypothesis is right this process should not be influenced (in any case not promoted) when IAA is supplied in abun-

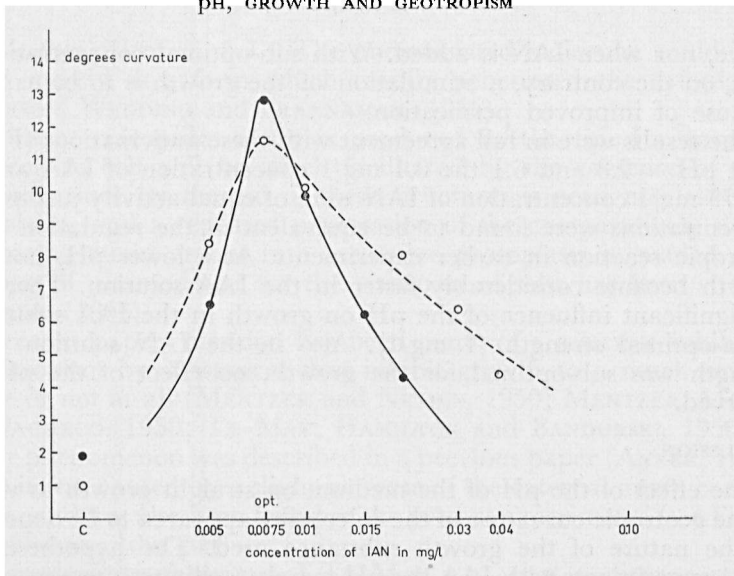


Fig. 3. The optimal IAN concentration for geotropism is not influenced by the acidity of the medium. ●—● pH = 4.1 ○—○ pH = 7.8.

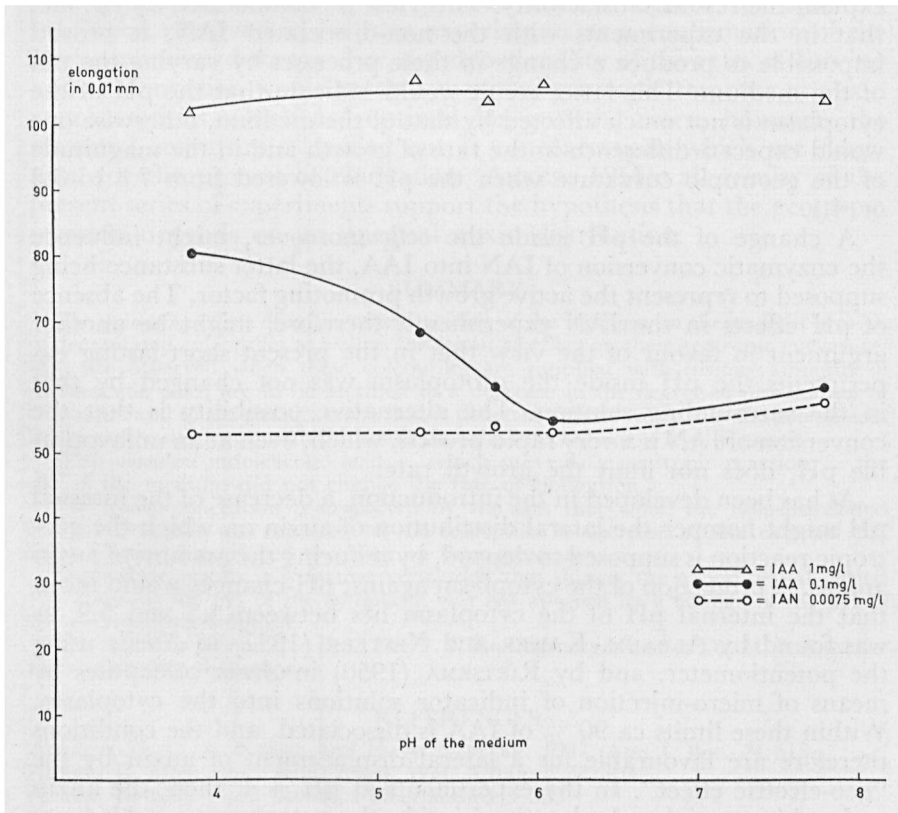


Fig. 4. Effect of the external pH on the straight growth at sub-optimal and supra-optimal IAA concentrations and at a sub-optimal IAN concentration.

dance, nor when IAN is added. With sub-optimal concentrations of IAA, on the contrary, a stimulation of the growth is to be expected because of improved permeation.

The results were in full agreement with these anticipations (Fig. 4).

At pH = 7.8 and 6.1 the 0.1 mg/l concentration of IAA and the 0.0075 mg/l concentration of IAN were of equal activity just as these concentrations were found to be equivalent in the regulation of the geotropic reaction in earlier experiments. At a lower pH, however, growth became considerably faster in the IAA solution. There was no significant influence of the pH on growth in the IAA solution of supra-optimal strength (1 mg/l). Also in the IAN solution whose strength was sub-optimal for the growth, no effect of the pH was observed.

DISCUSSION

The effect of the pH of the medium on straight growth as well as on the geotropic curvature of the coleoptiles appeared to be dependent on the nature of the growth substance used. The hypothesis that in the experiments with IAA the pH merely facilitates the penetration of this acid auxin by reducing the degree of dissociation, seems to explain the results satisfactorily. This view is corroborated by the fact that in the experiments with the non-dissociated IAN, it proved impossible to produce a change in these processes by varying the pH of the medium. This latter result would indicate that the pH of the cytoplasm is not much affected by that of the medium, otherwise one would expect a difference in the rate of growth and in the magnitude of the geotropic curvature when the pH is lowered from 7.8 to 3.8 or 4.1.

A change of the pH inside the cell, moreover, might influence the enzymatic conversion of IAN into IAA, the latter substance being supposed to represent the active growth promoting factor. The absence of pH effects in the IAN experiments, therefore, might be another argument in favour of the view that in the present short-lasting experiments the pH inside the protoplasm was not changed by that in the surrounding solution. The alternative possibility is that the conversion of IAN is a very rapid process, which, even at an unfavourable pH, does not limit the growth rate.

As has been developed in the introduction, a decrease of the internal pH might hamper the lateral distribution of auxin on which the geotropic reaction is supposed to depend, by reducing the amount of auxin anions. A protection of the cytoplasm against pH-changes would mean that the internal pH of the cytoplasm lies between 5.5 and 5.9, as was found by ALBAUM, KAISER and NESTLER (1937) in *Nitella* using the potentiometer, and by RIETSEMA (1950) in *Avena* coleoptiles by means of micro-injection of indicator solutions into the cytoplasm. Within these limits ca 90 % of IAA is dissociated, and the conditions therefore are favourable for a lateral displacement of auxin by the "geo-electric effect". In the experiments at pH = 4, then, the auxin molecules, once they had arrived inside the cytoplasm, would disso-

ciate at this pH of ca. 5.7. "The undissociated molecules could have served merely as a form which readily penetrated the cytoplasm" (ERICKSON, WEDDING and BRANNAMAN (1955).

In intact coleoptiles auxin is produced in the tip. Its transport from cell to cell will be much facilitated by the presence of plasmodesms, since by the aid of these structures it need not leave the protoplasm, and a repeated penetration of the comparatively slower permeating auxin anions into successive protoplasts is evaded. The meaning of plasmodesms for the transport of salts has been stressed by ARISZ (1956).

In recent papers mention has been made of a selective inhibition of geotropism by substances that inhibit growth to a much smaller degree or not at all (MENTZER and NETIEN, 1950; MENTZER, MOLHO and PACHECO, 1950; TE-MAY, HAMILTON and BANDURSKI, 1956). A similar phenomenon was described in a previous paper (ANKER, 1956), where it was caused by a slight increase of the external auxin concentration, and has also been found in the present experiments (see Fig. 1) after lowering the pH.

In contrast to the view of TE-MAY *et al.*, that a disproportional inhibition of geotropism and growth requires a modification of the CHOLODNY-WENT-theory, the present author regards the selective inhibition observed in his own experiments as a confirmation of the above-mentioned theory. For, a proportionality of the rates of curving and of elongation would mean a maximal geotropic response at auxin concentrations which are optimal for growth. Since, however, the maximal curvature is carried out at concentrations which are *suboptimal* for the process of elongation (ANKER, 1956), all results of the present series of experiments support the hypothesis that the geotropic reaction depends on an unequal auxin distribution.

SUMMARY

The *promoting* effect of an increased acidity of the medium on the straight growth of decapitated coleoptiles as well as the *inhibiting* effect on their geotropic curvature, that are observed when these coleoptiles are supplied with *limiting* amounts of indoleacetic acid, are to be ascribed to a decrease in the degree of dissociation of this auxin and to the circumstance that it penetrates more easily into the protoplasm in the molecular form, with the result that the internal auxin level is raised.

With *abundant* indoleacetic acid — which prevents a geotropic reaction — the pH of the medium did not change the rate of elongation.

The above conclusion is supported by the fact that when the non-dissociated indoleacetonitrile was supplied to the coleoptiles a decrease of the external pH from 7.8 to 3.8 produced no effect on these processes.

From these results it is further concluded that during the experimental period (75 minutes) the pH of the cytoplasm was not affected by a change of the pH of the surrounding solution.

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