In contrast to this, Zn^{++} ions at the same concentration inhibit its activity by approximatively 80%. At about five-fold higher concentration of Mn^{++}, the enzyme inhibited by zinc ions regains its original activity, indicating competition between the two ions. Other bivalent metal ions possess non-specific action on the enzyme activity, either inhibition (Ca^{++}, Cu^{++}) or activation (Mg^{++}, Fe^{++}, Co^{++}), but only to a slight degree. Phosphate ions in a concentration of 0.1 *M* decrease the enzyme activity by 70%. In the presence of Versene (0.02 *M*), activity is lowered to 1/7 of that originally present. The optimum pH in the presence of Mn^{++} ions lies at about pH 9.0, and at pH 8.5 in the absence of the activator.

During electrophoresis on a starch block at pH 8.6 diesterase moves toward the anode, indicating the acid character of the enzyme. Most of the inactive proteins are separated from the diesterase activity.

The enzyme is relatively stable at alkaline pH. A loss of approximatively 30% of its activity occurs when it is stored in 0.1 *N* NaOH at 0°, and 80% at 37° during 1 h. In an acid solution (pH less than 4.0) the enzyme is quickly inactivated. It is also thermolabile, losing 4/5 of its original activity when heated to 70° at pH 6.5 for 5 min. Appreciable loss of activity does not take place when the partially purified enzyme is stored in the frozen state (−12°) for several months and repeatedly thawed and frozen.

Phosphodiesterase causes hydrolysis of yeast RNA, RNA isolated from *Th. thioparus* cells (RNA-Th) by phenol extraction[7], DNA from the thymus (commercial) and the 'core' of yeast RNA obtained by exhaustive digestion of RNA with pancreatic ribonuclease[8]. If one of the above-mentioned highly polymerized polynucleotides is used as the substrate, Mn^{++} ions do not activate the enzyme. The fastest rate of hydrolysis is observed with RNA-Th, and the slowest with the 'core'. The enzyme also digests synthetic polynucleotides[9] such as polyadenylic acid (poly A) and other purine and pyrimidine 3′,5′-oligo-nucleotides. The hydrolysate of poly A was subjected to chromatography on DEAE-cellulose column[10]. The elution pattern of the hydrolysis products present is shown in Figure 2a. As the main products, adenosine (46%) and adenosine-5′-phosphate (31%) were found besides small amounts of oligonucleotides. The adenosine is the result of the presence of 5′-nucleotidase in the preparation of diesterase, as mentioned before. Adenosine and adenosine-5′-phosphate were identified by paper chromatography in solvent system: ethanol-ammonium acetate[11] and Rf values in Whatman 3 MM paper were 0.70 and 0.21 respectively. Adenosine-5′-phosphate, recovered from the column when incubated with 5′-nucleotidase isolated from the cells of *Th. thioparus*, was quantitatively transformed to adenosine (Figure 2b). Adenosine-3′-phosphate was completely resistant to this enzyme.

The fraction emerged from the column at higher concentrations of ammonium bicarbonate (labelled (pA)x in the Figure 2a) was first chromatographed on paper in the above solvent system (the Rf was determined to be 0.09), eluted from the paper, incubated with purified snake venom diesterase[12] and then rechromatographed under the same conditions. The spot of adenosine-5′-phosphate was only found (Figure 2b). Because the snake venom diesterase is almost without activity on oligonucleotides with 3′-phosphomonoester end groups[13], it must be assumed that oligonucleotides formed by diesterase from *Th. thioparus* are terminated with 5′-phosphomonoester end groups.

From results reported above it can be concluded that the enzyme from *Th. thioparus* splits off the diester linkages of phosphoric acid in position 3′ of polynucleotide chain. During the initial stage of the enzymic action in the presence of an excess of substrate, chiefly 5′-mononucleotides and small amounts of oligonucleotides were found. Hence, it must be assumed that phosphodiesterase causes gradual degradation of polynucleotide liberating 5′-phosphonucleotides[14].

Investigations on further purification of the enzyme and its specificity are under way and will be published elsewhere.

Résumé. La phosphodiestérase a été isolée des cellules du *Th. thioparus* et partiellement purifiée. Cette enzyme hydrolise les polynucléotides jusqu'à 5′-mononucleotides. Elle est spécifiquement activée par les ions du Mn^{++} et inhibée par les ions du Zn^{++}. Son pH optimum est compris entre 8,5–9,0. L'enzyme est thermolabile et relativement résistante en milieu alcalin.

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Investigations on Auxin-Gibberellin Interaction on the Growth Mechanism of *Helianthus annuus* Seedlings

The problem of auxin-gibberellin interaction on the mechanism of growth process could be discussed from two different points of view. The aim of the present research is to verify the validity of these two ideas and hence to explain the nature of this interaction on the growth of *Helianthus annuus* seedlings.

Method. The germinating achenes of *Helianthus* were sown in glass tubes containing a mixture of soil and washed sand; these were placed on a vertical klinostat under daylight. After a period of 6 days, the straight hypocotyls, of length approximately 5 cm, were selected to be decapitated. The decapitation was done exactly from the base of the cotyledons which are known to serve as auxin sources to the developing plant. These seedlings were then left in complete darkness for a period of 10 h, to ensure the thorough removal of all endogenous auxins. After this procedure, capillary tubes of length 2.5 cm containing 10^{-5} Mol isomolecular solutions of indole acetic acid (IAA), naphtalene acetic acid (NAA) and gibberellin (GB) respectively, were placed on the apex of the hypocotyls. As control sets, hypocotyls bearing capillary tubes with distilled water were used. For the measurements of the combined action of GB with IAA and NAA, a mixture of equal volumes of isomolecular (10^{-5} Mol) solutions of

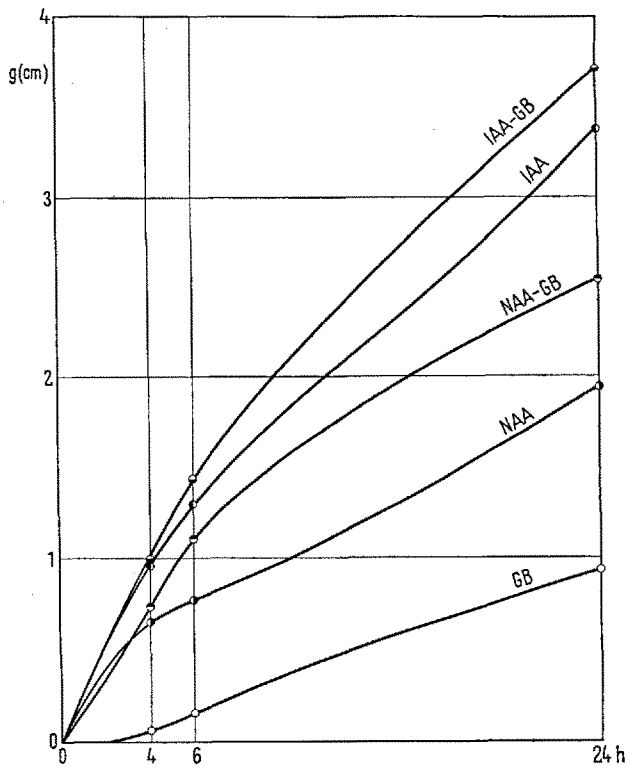
IAA-GB and NAA-GB were applied. The plants were placed close in front of a screen of Eidinger paper and were left in complete darkness at room temperature (24 ± 2°C) and relative humidity 78% for the whole of the experimental period. After having noted the initial height of the hypocotyls, measurements were taken under red light on the 4th, 6th, and 24th h, as indicated by the change in level of the reflection on the screen of the base of the capillary tubes. The values obtained were plotted against time as abscissa and straight growth as ordinate, the growth being measured as relative values of the control set. A measure of significance was performed for each exposure time in each series.

Results. The individual and the combined actions of GB with auxins are summarized in the Figure.

As the Figure demonstrates, the maximum growth reaction was produced by IAA which showed the steepest gradient compared with the other two growth regulators, GB produced a minimum action and NAA was in between. After having obtained these results, the auxin-gibberellin interaction on the same experimental object was analysed.

As seen from the Figure, the IAA-GB graph was more pronounced than the NAA-GB, yet the IAA-GB effect was nearly the same as that of IAA, for the values of both these curves do, in general, correspond. The statistical calculations revealed that the values of IAA-GB and IAA respectively were insignificant. The NAA-GB graph and the NAA-curve followed a similar path up to the 4th h, henceforth the NAA-GB graph diverged strongly to follow a parallel path with the NAA-curve but on a higher level, in accordance with the significance of the NAA-GB and NAA values after the 4th h.

Discussion. The effect of auxins and gibberellin on the growth process of plants has been subjected to much detailed research, and it was concluded that GB alone had very little effect on this process. BRIAN and HEMMING[1–3], by applying a GB solution on the shoot of pea seedlings, found an increase in growth. KUSE[4], in his experiments on debladed petioles of Ipomoea Batatas, came to the conclusion that GB applied alone produced a very slight growth, this being in good agreement with the findings of the present research. On the other hand, WAREING[5], by applying GB on the cambial regions of woody dicotyledons, noticed very little cambial activity. This limited action of the compound on the cambial region also explains the small effect of GB on growth. As already known in the relevant literature, in the present research too, the application of NAA and especially of IAA increased the growth.



The effect of IAA, NAA, GB, and IAA-GB, NAA-GB on the straight growth of *Helianthus annuus* seedlings. Abscissa: time in h and growth of control set. Ordinate: straight growth in cm.

Having observed the sole effect of GB on the growth process, the auxin-gibberellin interaction attracted the attention of several research workers. Among them, HAYASHI and MURAKAMI[6], by treating IAA-extracts of several different plants with GB, could find no increase in the conversion of tryptophan into IAA. PILET[7] noticed the decrease in the IAA oxidase activity *in vivo*, under the effect of GB; hence he concluded that GB acts on the growth process by eliminating the IAA oxidase destruction. BRIAN and HEMMING[1–3] observed that, when GB is applied to pea internodes, the growth produced is the same as that of untreated plants. Hence, GB can only act in the presence of an auxin such as IAA in the ambient medium. KUSE[4], similar to the latter investigators, noticed that GB promotes growth only in the presence of IAA. On the other hand, according to PURVES and HILLMAN[8], the primary effects of GB and IAA are completely independent of one another, and when applied together their combined action is only partly additive. According to these different observations, the two concepts which could explain the auxin-gibberellin interaction are that: either GB acts through an auxin-mediated medium, or else the action of GB is independent of other growth regulating systems. The results of the present paper show that the auxin-gibberellin interaction cannot be interpreted fully with either of these two concepts, but that the effect of GB depends on the nature of the auxins. This is clearly shown in the results obtained, namely that whilst GB added to IAA showed practically no different effect from IAA alone, yet GB added to NAA was significantly additive compared to NAA after the 4th h[9].

Résumé. L'auteur a étudié l'action combinée des gibberellines et des auxines chez l'hypocotyle de l'*Helianthus annuus*. L'action de AIA + GB n'a pas été différente de l'action de l'AIA seule, tandis que l'ANA+ GB a eu un effet promotif que n'avait pas l'ANA seule. D'où l'on peut déduire que cette action combinée dépend de la nature de l'auxine.

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