EFFECTS OF ADENOSINE TRIPHOSPHATE AND OTHER SUBSTANCES ON HYDROXYPROLINE-INDUCED INHIBITION OF *AVENA* COLEOPTILE ELONGATION

THESIS

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By

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CHAPTER I

INTRODUCTION

It has been known for some time that hydroxyproline, a structural analogue of proline, has the ability to inhibit growth of plant tissues. Steward *et al.* discovered that hydroxyproline reduced growth of carrot tissue explants and that proline reversed its effect.[1] Cleland found that proline effectively reduced the hydroxyproline-induced inhibition of *Avena* coleoptile elongation.[2]

Although the exact mechanism of hydroxyproline action is not clearly understood at the present time, there have been numerous reports of the role of proline and hydroxyproline in various plant tissues. Steward *et al.* have reported that protein in carrot tissue has a high hydroxyproline content.[3] Since Pollard and Steward have found that exogenous hydroxyproline is not incorporated to any appreciable extent into

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[1] F. C. Steward, *et al.*, "The Effects of Selected Nitrogen Compounds on the Growth of Plant Tissue Cultures," *Biochimica et Biophysica Acta*, XXVIII (1958), 308-317.

[2] R. Cleland, "Hydroxyproline as an Inhibitor of Auxin-Induced Cell Elongation," *Nature*, CC (1963), 908-909.

[3] Steward, *et al.*, *op. cit.*

carrot protein,[4] the presence of hydroxyproline in carrot protein must result from the conversion of some substance already incorporated into the protein. Steward et al. discovered that some proline is converted to hydroxyproline after proline is incorporated into carrot tissue protein.[5]

The results of Steward et al.[6] are substantiated by workers using plant tissues other than carrot explants. Lamport reported that hydroxyproline was found in the protein of sycamore[7] and bean cells.[8] Lyndon and Steward reported that some proline was converted to hydroxyproline after proline had been incorporated into potato-cell protein.[9] Olsen found that the hydroxylation occurred after proline

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[4]J. K. Pollard and F. C. Steward, "The Use of C^{14}-Proline by Growing Cells; Its Conversion to Protein and to Hydroxyproline," Journal of Experimental Botany, X (1959), 17-32.

[5]F. C. Steward, et al., "Nitrogen Metabolism, Respiration, and Growth of Cultured Plant Tissue," Journal of Experimental Botany, IX (1958), 11-49.

[6]Ibid.

[7]D. T. A. Lamport, "Primary Cell Wall Protein; Structure and Metabolism," Plant Physiology, XXXVII suppl. (1962), xvi.

[8]D. T. A. Lamport and D. H. Northcote, "Hydroxyproline in Primary Cell Walls of Higher Plants," Nature, CLXXXVIII (1960), 665-666.

[9]R. F. Lyndon and F. C. Steward, "The Incorporation of ^{14}C-Proline into the Proteins of Growing Cells," Journal of Experimental Botany, XIV (1963), 42-55.

was incorporated into protoplasmic protein of tobacco explants and before its deposition in the cell wall.[10]

Cleland discovered that the addition of hydroxyproline to oat coleoptile sections caused a slight decrease in incorporation of proline into protein and that some hydroxyproline could be directly incorporated into protein.[11] Since Pollard and Steward have reported that there is no significant incorporation of exogenous hydroxyproline into carrot protein,[12] it would seem that the mechanism of hydroxyproline inhibition in oat and carrot tissues might be different.

Villa-Trevino et al. reported that ethionine, an antagonist of methionine, inhibited protein synthesis in rat liver by trapping ATP.[13] Norris found that ATP reversed ethionine-induced inhibition of Avena coleoptile elongation

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[10] A. C. Olson, "Proteins and Plant Cell Walls, Proline to Hydroxyproline in Tobacco Suspension Cultures," Plant Physiology, XXXIX (1964), 543-550.

[11] R. Cleland, "Possible Mechanisms of Inhibition by Hydroxyproline of Auxin-Induced Growth," Plant Physiology, XLI suppl. (1966), xlvi.

[12] Pollard and Steward, op. cit.

[13] S. Villa-Trevino, et al., "The Role of Adenosine Triphosphate Deficiency in Ethionine-Induced Inhibition of Protein Synthesis," Journal of Biological Chemistry, CCXXXVIII (1963), 1757-1763.

and suggested that ethionine inhibits protein synthesis of oat segments by interfering with ATP metabolism.[14] Since ethionine and hydroxyproline are both structural analogues of amino acids, it was thought that the mechanism of hydroxyproline inhibition of oat coleoptile elongation might involve ATP metabolism.

This paper presents the results of an investigation of the effects of hydroxyproline on the elongation of Avena coleoptile sections. Data are presented which suggest that hydroxyproline inhibition of growth may involve ATP metabolism.

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[14] W. E. Norris, Jr., "Reversal of Ethionine-Induced Inhibition of Elongation of Avena Coleoptiles by Adenosine Triphosphate," Archives of Biochemistry and Biophysics, CVII (1964), 352-355.

CHAPTER II

MATERIALS AND METHODS

The experimental procedures were identical to those described by Norris.[1] *Avena sativa* L. seeds, Victory strain (U.S. Department of Agriculture, C.I. 2020), were used in these experiments. The seedlings were grown on filter paper strips that were immersed in distilled water which previously had been aerated. Additional details of the growing method are described by Wiegand and Schrank.[2] Only seedlings that were 72 hours old and that had 30 ± 2 mm. coleoptiles were used.

Growth measurements were made on coleoptiles which were isolated from the seeds and primary leaves. The second 5 mm. sections at the apical end were used. These sections were floated in Petri dishes containing the media (20 sections/ 20 ml. solution). During cutting and transferring, the sections were exposed to red light of wavelengths longer than 6074 Å. Wiegand and Schrank observed that wavelengths

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[1] W. E. Norris, Jr., "Reversal of Ethionine-Induced Inhibition of Elongation of *Avena* Coleoptiles by Adenosine Triphosphate," *Archives of Biochemistry and Biophysics*, CVII (1964), 352-355.

[2] C. F. Wiegand and A. R. Schrank, "Regimen for Growing Uniform *Avena* Coleoptiles," *Botanical Gazette*, CXXI (1959), 106-110.

in this portion of the spectrum had no effect on the elongation of floating sections.[3] The sections were allowed to elongate in the dark for 24 hours. At the end of this period, their lengths were measured with a micrometer in the ocular of a stereoscopic microscope. All procedures were carried out at a temperature of 22 ± 1°C.

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[3] Ibid., p. 107.

CHAPTER III

RESULTS

The inhibitory effect of various concentrations of hydroxyproline was first determined. Figure 1 shows that hydroxyproline in water inhibited elongation of Avena coleoptile sections. The elongation of sections incubated in hydroxyproline was compared to the amount of elongation which occurred in water. The average length of an Avena coleoptile section in water after 24 hours was 6.7 mm. The initial length of a segment was 5.0 mm.; thus, an elongation of 1.7 mm. was shown by the control section. A hydroxyproline concentration of 1×10^{-2}M caused 50% inhibition of elongation and was used as the standard concentration of inhibitor in subsequent experiments. Various concentrations of proline in water caused 10% to 20% more elongation than did water alone. Proline in concentrations ranging from 1×10^{-3}M to 1×10^{-2}M, when mixed with 1×10^{-2}M hydroxyproline, completely reversed its inhibitory influence. The amount of elongation occurring in the mixtures was 10% to 20% greater than in the water control.

The results shown in Figure 2 differ from Figure 1 only in that 1×10^{-5}M IAA was used as the control solution. The elongation of oat coleoptile sections was greatly increased

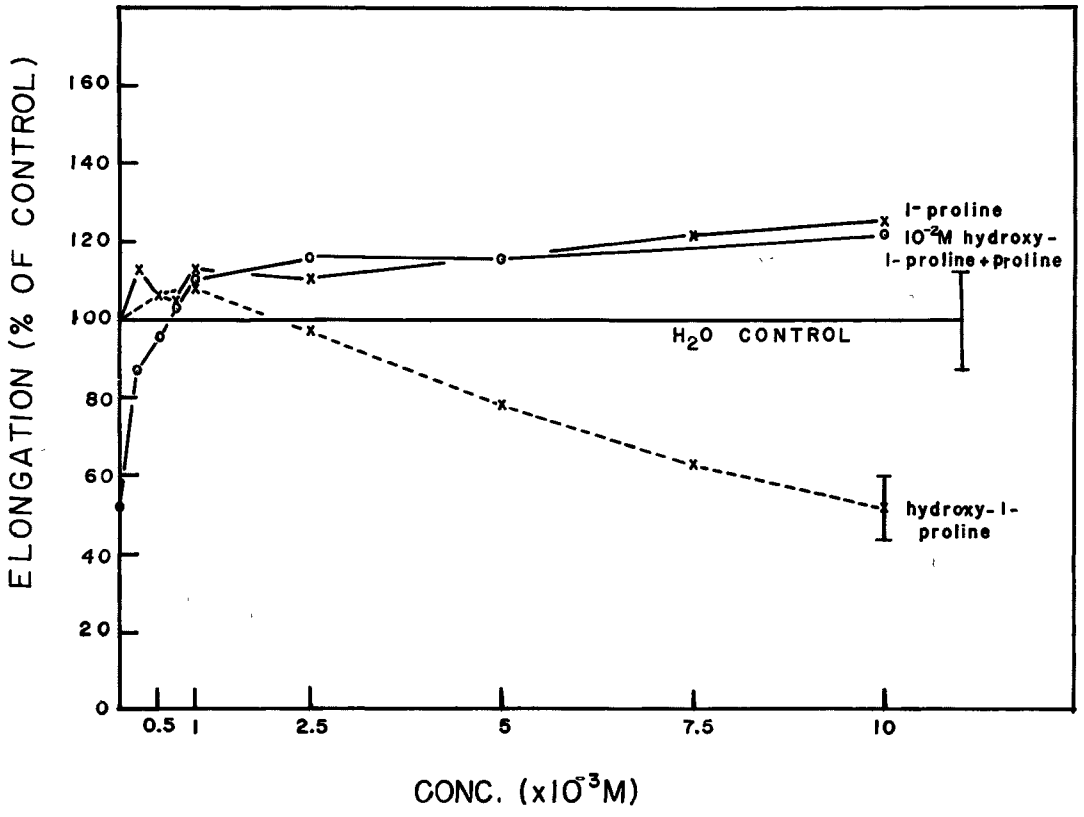

FIGURE 1

EFFECT OF HYDROXYPROLINE AND PROLINE ON AVENA COLEOPTILE ELONGATION IN WATER

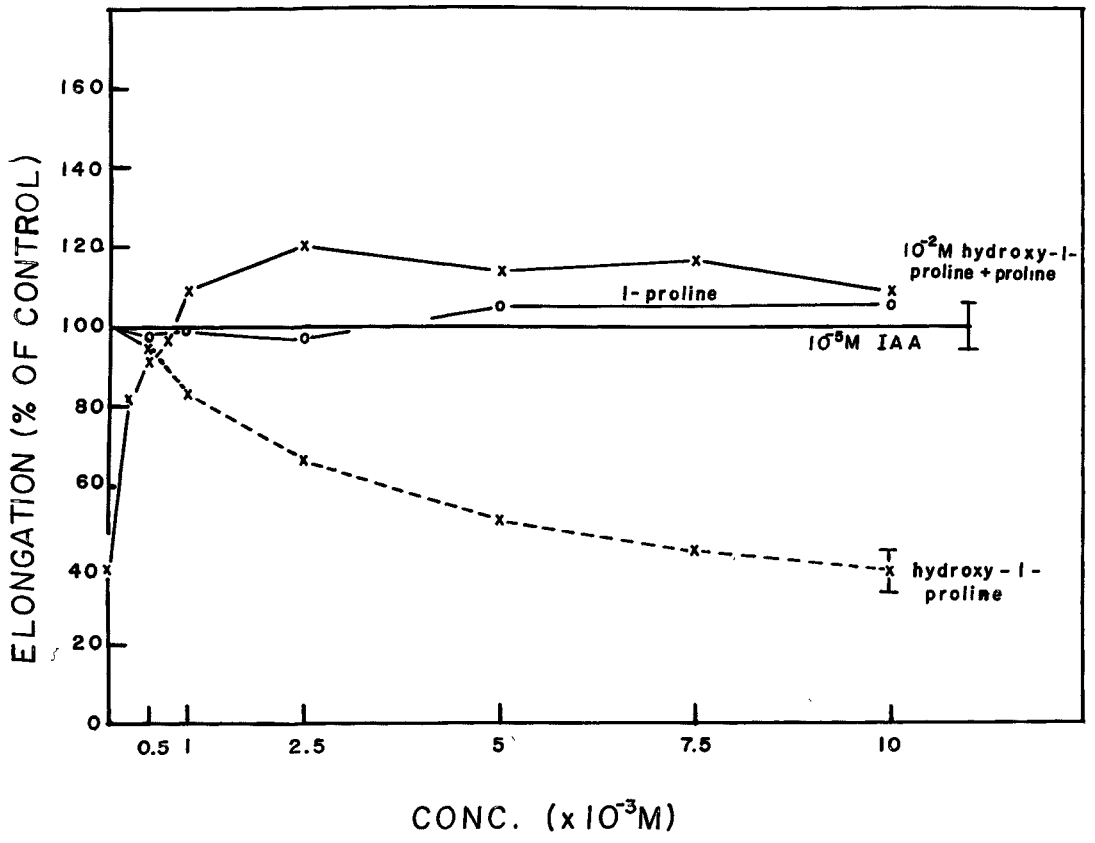

FIGURE 2

EFFECT OF HYDROXYPROLINE AND PROLINE ON AVENA COLEOPTILE ELONGATION IN IAA

in the presence of exogenous IAA. Hydroxyproline, at a concentration of 1 x 10^{-2}M, caused about 60% inhibition of auxin-induced elongation. Proline had less stimulating effect on growth in IAA than it did in water solutions. Elongation in proline solutions ranging from 5 x 10^{-3}M to 1 x 10^{-2}M was 10% greater than in the IAA control. Proline concentrations ranging from 1 x 10^{-3}M to 1 x 10^{-2}M in the presence of hydroxyproline and IAA completely reversed the inhibition and stimulated growth more than did the same proline concentrations in the absence of the inhibitor.

Cleland found that 4 x 10^{-5}M hydroxyproline inhibited oat coleoptile elongation 50% in the presence of IAA (5 ug./ml.), sucrose (2% wt./vol.), and potassium maleate 2.5 mM).[1] Auxin-induced growth was inhibited almost 100% by 1 x 10^{-3}M hydroxyproline. Proline completely reversed the hydroxyproline effect and had no influence on growth in the absence of hydroxyproline. The difference in the effective concentrations of hydroxyproline in this study and in Cleland's study[2] may be due to the presence of sucrose and potassium maleate in the culture media.

Figure 3 shows the influence of various concentrations of ATP on elongation in the presence or absence of 1 x 10^{-2}M

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[1]R. Cleland, "Hydroxyproline as an Inhibitor of Auxin-Induced Cell Elongation," Nature, CC (1963), 908-909.

[2]Ibid.

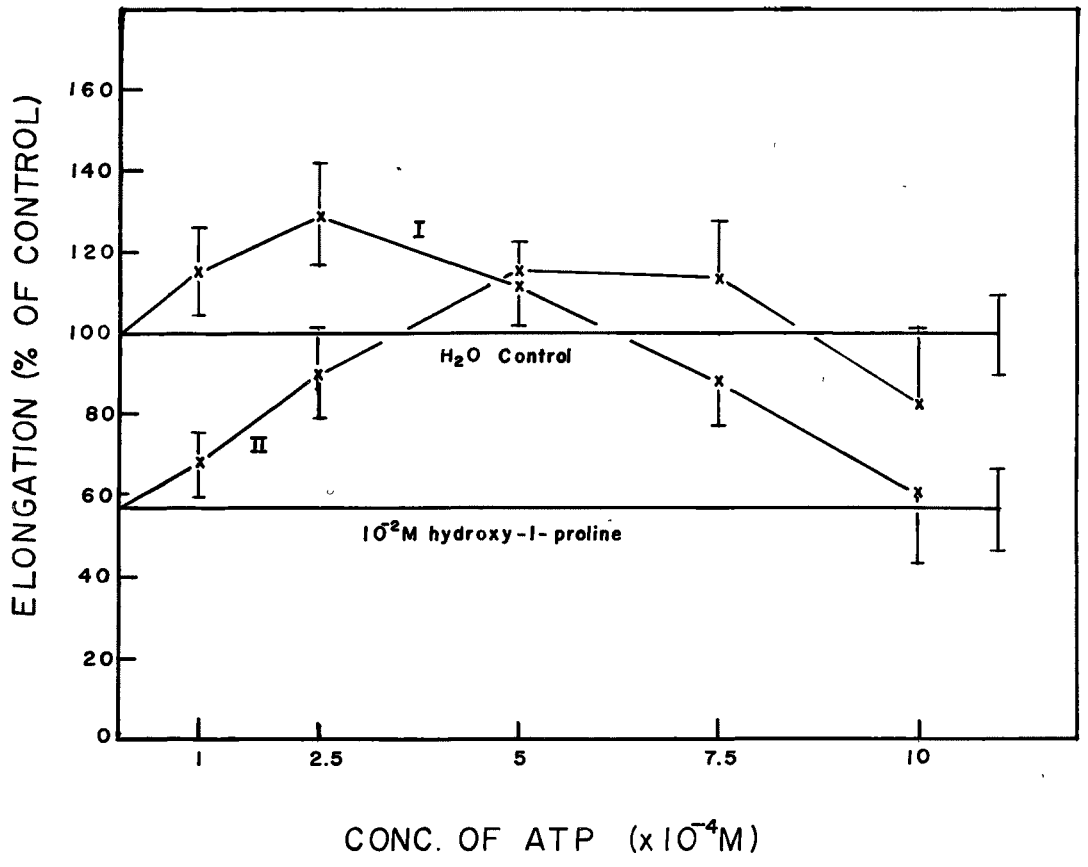

FIGURE 3

EFFECT OF ATP ON ELONGATION OF AVENA COLEOPTILE SECTIONS IN WATER

hydroxyproline with water as the control solution. Curve I indicates that the greatest stimulation of elongation of the coleoptile segments was obtained with 2.5×10^{-4}M ATP. At this ATP concentration, growth was increased 30% more than the water control value. Higher concentrations of ATP caused a marked reduction in elongation. Curve II shows the ability of ATP to reverse the hydroxyproline inhibition. Solutions of 5×10^{-4}M and 7.5×10^{-4}M ATP in the presence of 1×10^{-2}M hydroxyproline effectively reversed the inhibition and caused about 15% more growth than the water control.

Figure 4 shows that hydroxyproline inhibition of auxin-induced growth of coleoptile segments was decreased by about 15% upon the addition of 5×10^{-4}M ATP (Curve II). It is apparent upon comparison of Figures 3 and 4 that solutions of ATP in water exhibit a greater ability to reverse hydroxyproline inhibition than do solutions of ATP in IAA. The data presented in Curve I show that in the absence of hydroxyproline 1×10^{-4}M ATP increases elongation approximately 10% more than the IAA control. Higher concentrations of ATP had an inhibitory effect. The results presented in Figures 3 and 4 resemble data which show that ATP is effective in decreasing ethionine inhibition of *Avena* coleoptile elongation in water and in IAA.[3]

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[3] W. E. Norris, Jr., "Reversal of Ethionine-Induced Inhibition of Elongation of *Avena* Coleoptiles by Adenosine Triphosphate," *Archives of Biochemistry and Biophysics*, CVII (1964), 352-355.

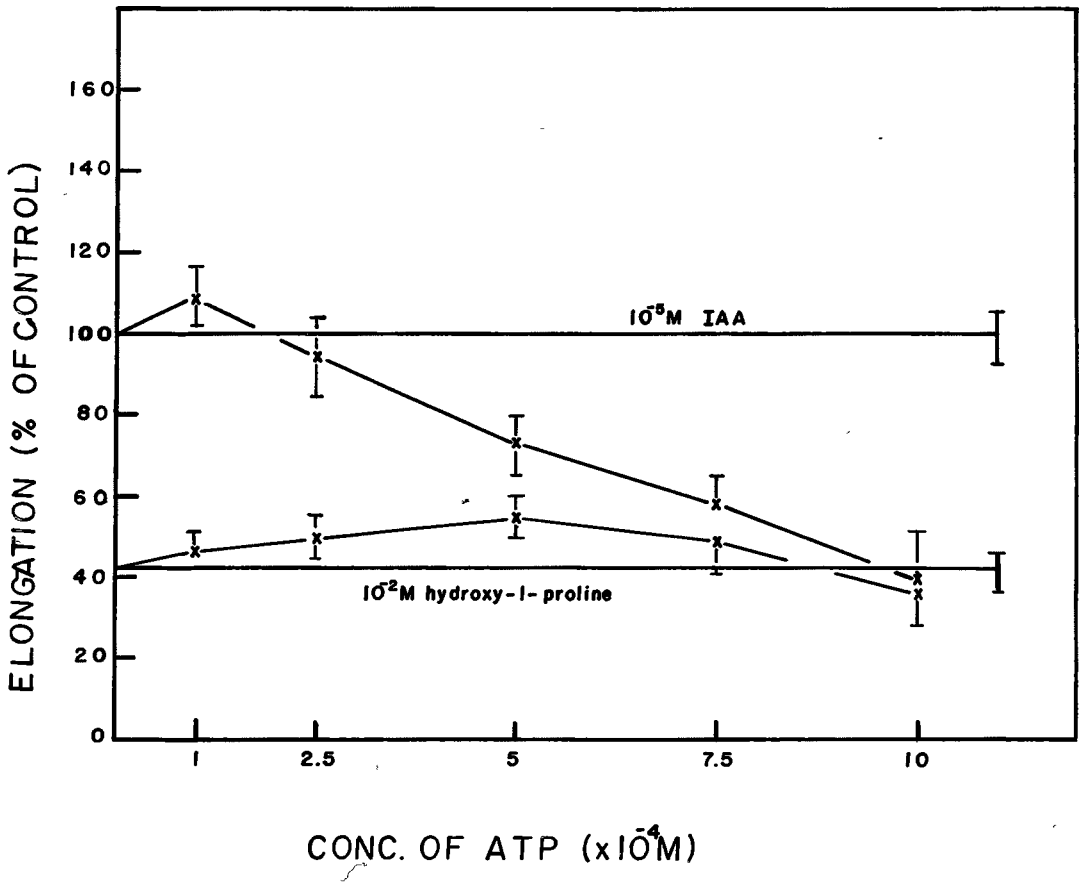

FIGURE 4.

EFFECT OF ATP ON ELONGATION OF AVENA COLEOPTILE SECTIONS IN IAA

A time course study was conducted to determine the initiation of inhibition of oat coleoptile elongation by selected concentrations of hydroxyproline in water and in IAA. These data are presented in Figure 5. The amount of elongation produced by 1×10^{-5}M IAA was 4.2 mm. after 24 hours. A hydroxyproline concentration of 1×10^{-4}M had little inhibitory effect in IAA. The inhibition of 1×10^{-3}M and 1×10^{-2}M hydroxyproline appeared during the first 3 hours. Hydroxyproline produced the greatest reduction of auxin-induced growth at a concentration of 1×10^{-2}M, although 1×10^{-3}M hydroxyproline showed some inhibition. A concentration of 1×10^{-4}M hydroxyproline had no effect on elongation in water; however, 1×10^{-3}M hydroxyproline reduced growth slightly. The greatest amount of inhibition was noted in a concentration of 1×10^{-2}M. The lag in appearance of hydroxyproline inhibition occurs only after a certain amount of auxin-induced elongation has taken place.[4]

Another time course experiment which indicates the influence of 1×10^{-2}M hydroxyproline in water, in IAA, and in IAA plus sucrose is shown in Figure 6. The amount of inhibition was increased in the presence of sucrose. These

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[4]R. Cleland, "Hydroxyproline as an Inhibitor of Auxin-Induced Cell Elongation," Nature, CC (1963), 908-909.

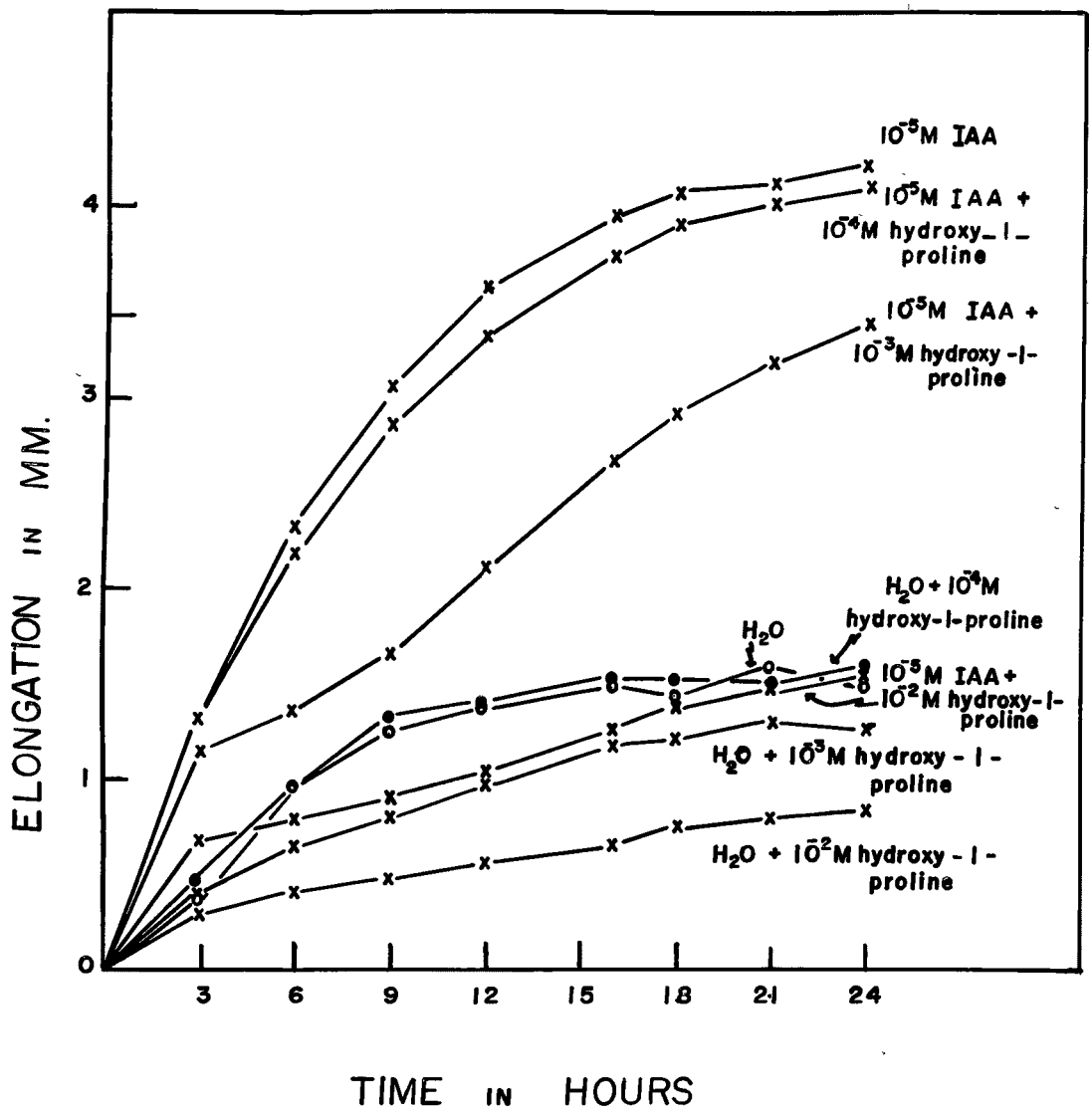

FIGURE 5

TIME COURSE OF HYDROXYPROLINE INHIBITION OF AVENA COLEOPTILE SECTIONS IN WATER AND IN IAA

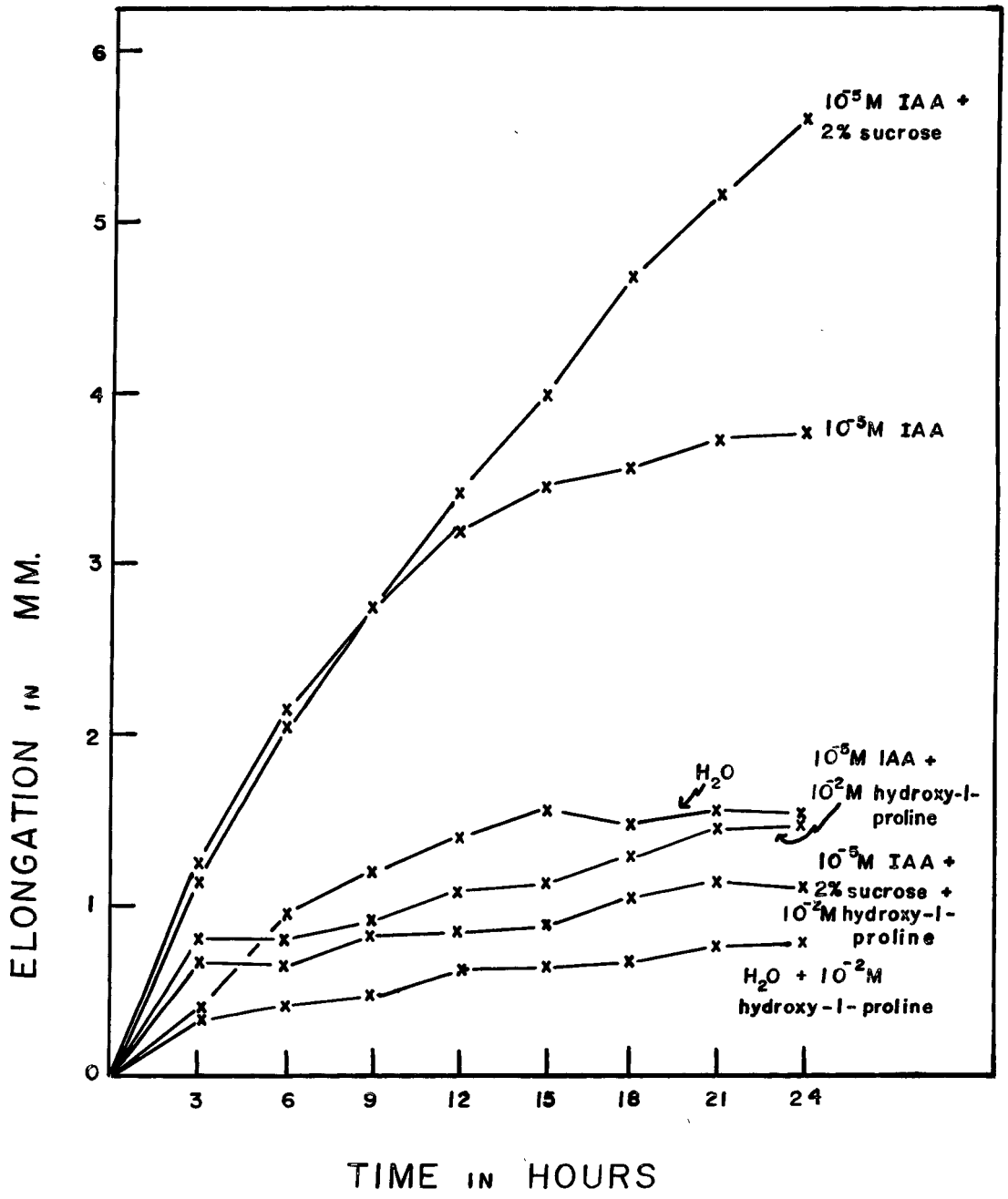

FIGURE 6

TIME COURSE OF HYDROXYPROLINE INHIBITION OF AVENA COLEOPTILE SECTIONS IN WATER, IN SUCROSE, AND IN IAA

data are in agreement with the results reported previously by Cleland.[5]

The influence of ATP on hydroxyproline inhibition in 2% sucrose and in 2% sucrose plus IAA is shown in Figures 7A and 7B. Figure 7A shows that the extent of ATP reversal of hydroxyproline inhibition with 2% sucrose as the control is similar to the ATP effect on inhibition in water, as shown in Figure 3. In both cases the hydroxyproline inhibition was completely reversed. An ATP concentration of 2.5×10^{-4}M stimulated coleoptile elongation more in a control solution of 2% sucrose than it did in water alone. The growth induced by 2.5×10^{-4}M ATP in 2% sucrose was 80% greater than the sucrose control. The results of various concentrations of ATP on elongation in 2% sucrose plus IAA in the presence and the absence of hydroxyproline are shown in Figure 7B. These data are very similar to the effects displayed by ATP in Figure 3. In both cases ATP decreased inhibition by 15% to 20%. In both Figures 3 and 7B the ATP influence on elongation in the absence of hydroxyproline reveals inhibition at higher concentrations.

The ability of glutamic acid to reverse hydroxyproline inhibition of coleoptile elongation in water and in IAA is shown in Figures 8A and 8B. Glutamic acid concentrations in

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[5]R. Cleland, "Inhibition of Cell Elongation in Avena Coleoptile by Hydroxyproline," Plant Physiology, XLII (1967), 271-274.

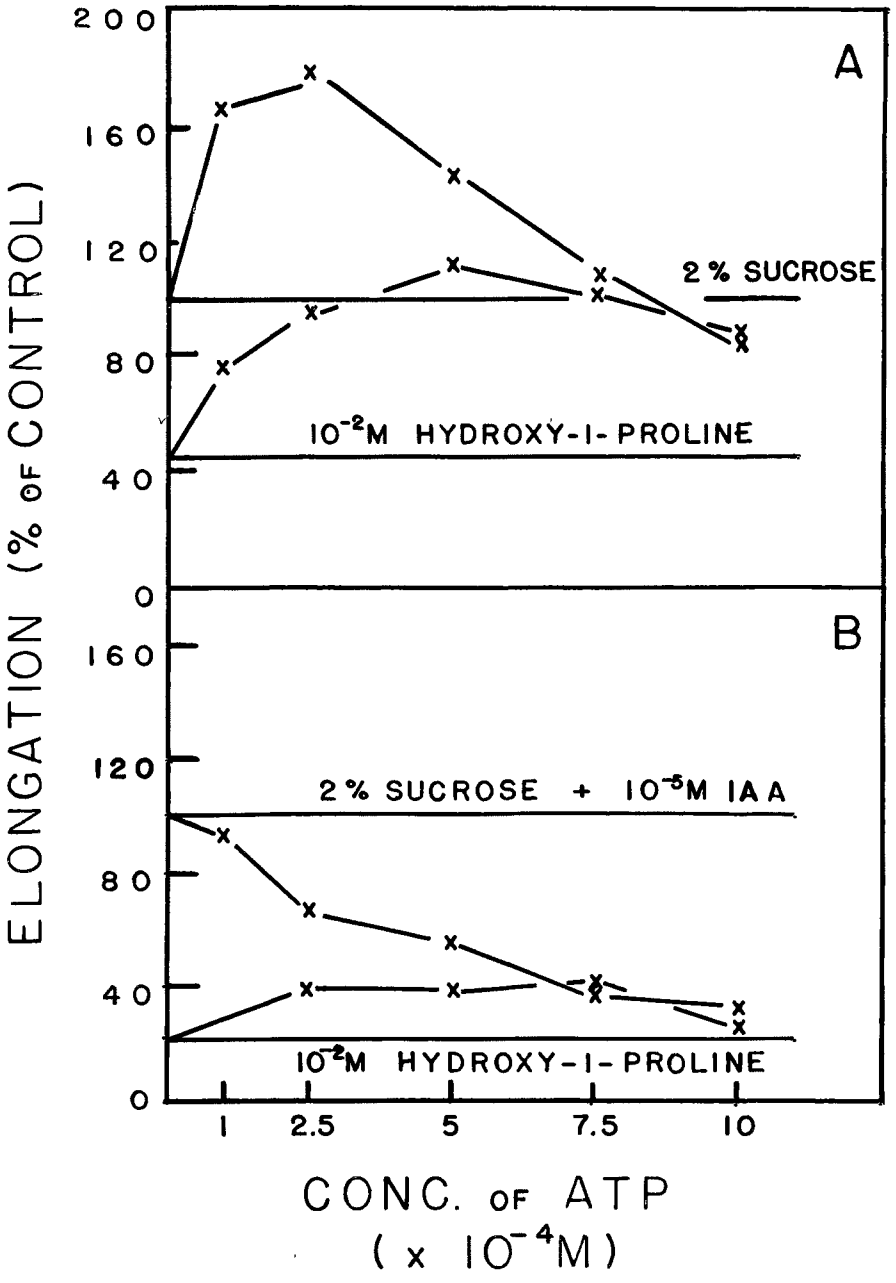

FIGURE 7

EFFECT OF ATP ON AVENA COLEOPTILE ELONGATION IN SUCROSE AND IN SUCROSE PLUS IAA

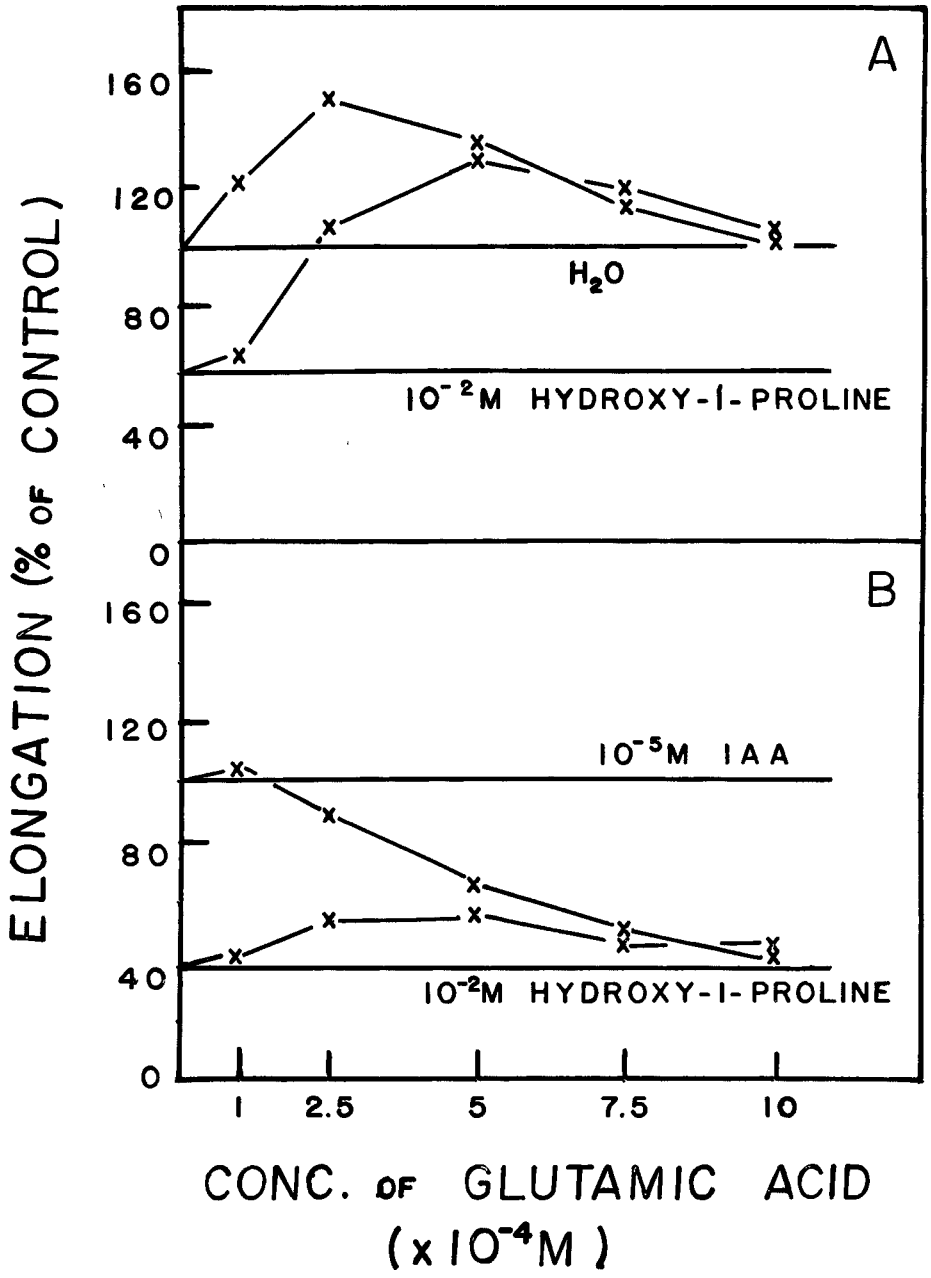

FIGURE 8

EFFECT OF GLUTAMIC ACID ON AVENA COLEOPTILE ELONGATION IN WATER AND IN IAA

water ranging from 2.5×10^{-4}M to 1×10^{-3}M completely reversed the hydroxyproline effect. The same concentrations of glutamic acid in IAA decreased inhibition by 15% to 20%. A 2.5×10^{-4}M glutamic acid solution in the absence of hydroxyproline caused 50% more growth than the water control. No significant enhancement of coleoptile elongation was noted in IAA solutions of glutamic acid. These curves showing the effects of glutamic acid on Avena coleoptile elongation are similar to those resulting from ATP.

Figures 9A and 9B indicate that ornithine monohydrochloride slightly decreased hydroxyproline inhibition of elongation of oat sections. Figure 9B shows that a 20% reduction of hydroxyproline inhibition was obtained in 7.5×10^{-4}M ornithine in IAA. Maximum reversal of inhibition in water was obtained by 1×10^{-3}M ornithine, as shown in Figure 9A. Ornithine stimulated growth 20% to 30% in water and about 15% in IAA. These data support results of an earlier investigation by Cleland in which he noted that ornithine and glutamic acid in IAA possessed the ability to decrease hydroxyproline inhibition.[6]

The influence of guanine hydrochloride upon hydroxyproline inhibition is shown in Figures 10A and 10B. Figure 10A shows

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[6] Ibid.

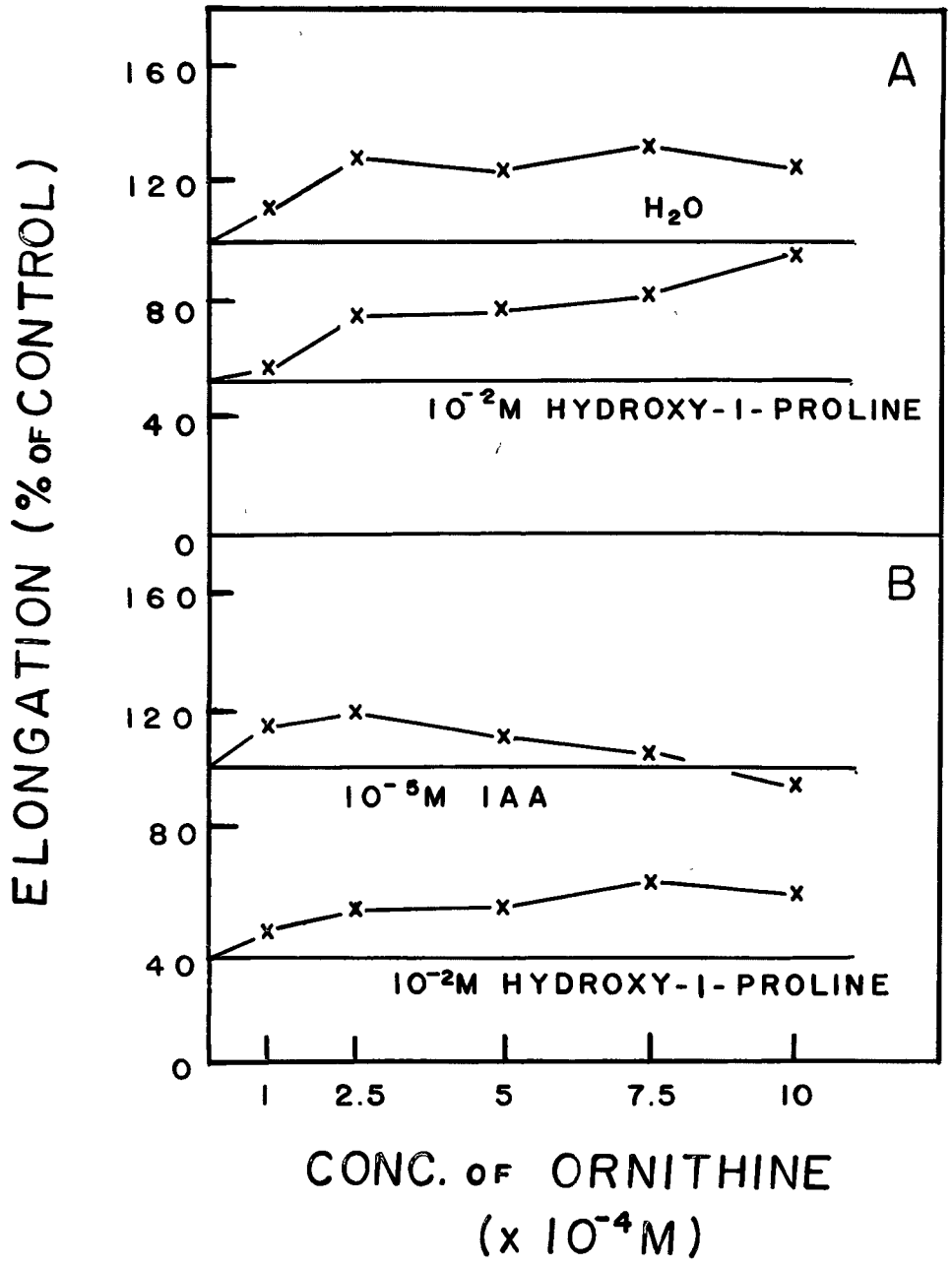

FIGURE 9

EFFECT OF ORNITHINE ON AVENA COLEOPTILE ELONGATION IN WATER AND IN IAA

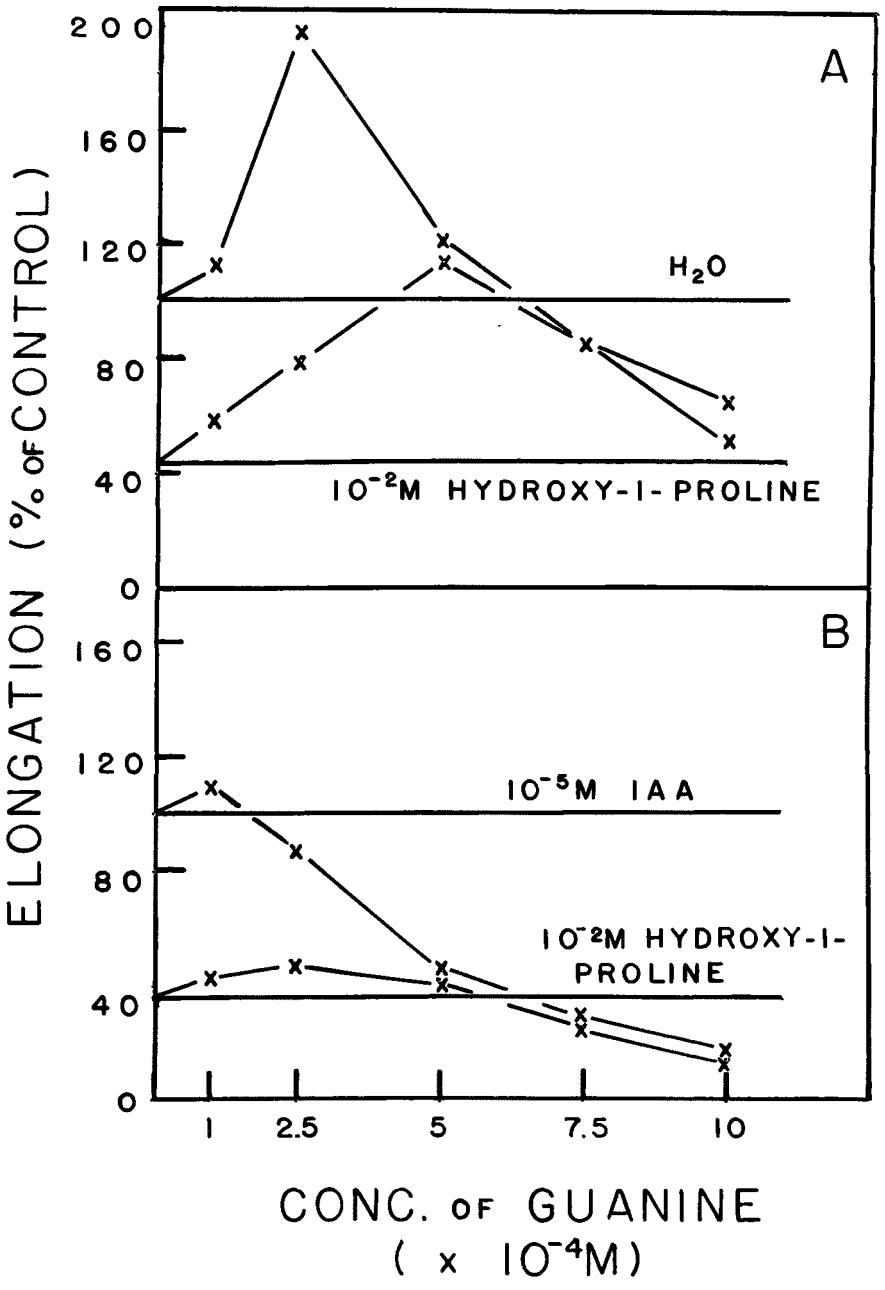

FIGURE 10

EFFECT OF GUANINE ON AVENA COLEOPTILE ELONGATION IN WATER AND IN IAA

that a 2.5×10^{-4}M guanine solution in the absence of hydroxyproline increased elongation nearly 100% above the control value. Complete reversal of hydroxyproline inhibition was obtained with 5×10^{-4}M guanine. Guanine had little effect on Avena coleoptile growth in IAA, as shown in Figure 10B. Various concentrations of guanine decrease hydroxyproline inhibition by only 10%. Guanine at a concentration of 1×10^{-4}M caused 10% more growth than did IAA. Higher concentrations of guanine had an inhibitory effect.

Table I shows the influence of other compounds on Avena coleoptile elongation in the presence of 1×10^{-2}M hydroxyproline in water or in IAA. None of the substances tested displayed any substantial reversal effect on the inhibition of elongation in IAA. Some reversal was noted in water solutions. The greatest reversal of the hydroxyproline inhibition was produced by 2.5×10^{-4}M adenine sulfate which reduced the hydroxyproline-induced inhibition of elongation by more than 55% in water.

TABLE I

ABILITY OF VARIOUS COMPOUNDS TO REVERSE HYDROXYPROLINE-INDUCED INHIBITION OF OAT COLEOPTILE ELONGATION IN WATER AND IN IAA

	Maximum Reversal (Concentration)		% Reversal	
Substance Tested	In Water	In IAA	In Water	In IAA
Adenine	5×10^{-5}M	...	14%	...
Adenine sulfate	2.5×10^{-4}M	1.25×10^{-4}M	55%	14%
Adenosine	1×10^{-3}M	...	9%	...
Arginine	7.5×10^{-4}M	...	15%	...
Cytosine	5×10^{-4}M	...	11%	...
Guanosine	...	...	...	...

CHAPTER IV

DISCUSSION

The results of this study indicate that 1×10^{-2}M hydroxyproline is an effective inhibitor of *Avena* coleoptile elongation and that proline completely reverses this hydroxyproline inhibition. These data are in agreement with the reports of Cleland[1] and Steward *et al.*[2] The exact mechanism by which hydroxyproline inhibits elongation is not clearly understood at the present time.

A possible mode of hydroxyproline action may involve the incorporation of hydroxyproline into protein in place of proline. Cleland reported that free hydroxyproline could be incorporated directly into protein and suggested that the resulting abnormal protein might be functional in growth inhibition.[3] Perusal of the literature reveals evidence in support of a mechanism of this type. Munier and Cohen have shown that structural analogues can be incorporated into

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[1] R. Cleland, "Hydroxyproline as an Inhibitor of Auxin-Induced Cell Elongation," *Nature*, CC (1963), 908-909.

[2] F. C. Steward, *et al.*, "The Effects of Selected Nitrogen Compounds on the Growth of Plant Tissue Cultures," *Biochimica et Biophysica Acta*, XXVIII (1958), 308-317.

[3] R. Cleland, "Possible Mechanisms of Inhibition by Hydroxyproline of Auxin-Induced Growth," *Plant Physiology*, XLI suppl. (1966), xlvi.

protein in place of amino acids.[4] Gross and Tarver found that ethionine was incorporated into protein in place of methionine.[5] Munier and Cohen also reported that the amino acid analogues, norleucine and fluorophenylalanine, bring about synthesis of abnormal protein.[6]

Stetten[7] and Pollard and Steward[8] have reported that no exogenous hydroxyproline is incorporated into protein. These data differ from the results reported by Cleland.[9] The explanation might possibly be in the concentrations of hydroxyproline used. Low concentrations of exogenous hydroxyproline might not be sufficient to compete with the

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[4]R. Munier and G. N. Cohen, "Incorporation of Structural Analogues of Amino Acids into the Bacterial Proteins during Their Synthesis in vivo," Biochimica et Biophysica Acta, XXXI (1959), 379-390.

[5]D. Gross and H. Tarver, "Studies on Ethionine IV. The Incorporation of Ethionine into the Proteins of Tetrahymena," Journal of Biological Chemistry, CCXVII (1955), 169-182.

[6]Munier and Cohen, op. cit.

[7]M. R. Stetten, "Some Aspects of the Metabolism of Hydroxyproline Studied with the Aid of Isotopic Nitrogen," Journal of Biological Chemistry, CLXXXI (1949), 31-37.

[8]J. K. Pollard and F. C. Steward, "The Use of C^{14}-Proline by Growing Cells; Its Conversion to Protein and to Hydroxyproline," Journal of Experimental Botany, X (1959), 17-32.

[9]R. Cleland, "Possible Mechanisms of Inhibition by Hydroxyproline of Auxin-Induced Growth," Plant Physiology, XLI suppl. (1966), xlvi.

cellular concentration of proline for a place in protein, whereas higher concentrations of hydroxyproline might result in its incorporation into protein to form abnormal protein. Another possible explanation of these data might be that hydroxyproline inhibits growth of different plant tissues by different mechanisms. The results presented in this study show that glutamic acid and ornithine partially reversed hydroxyproline inhibition of auxin-induced growth. These data are in agreement with the findings of Cleland.[10] The metabolic relationship between glutamic acid, ornithine, and proline has been reported by Stetten[11] and Stetten and Schoenheimer.[12] They found that exogenous glutamic acid or ornithine could be converted to proline. Thus, the increased concentration of proline could reverse the inhibition induced by hydroxyproline.

Cleland also reported that arginine partially reduced hydroxyproline inhibition of auxin-induced growth.[13]

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[10] R. Cleland, "Inhibition of Cell Elongation in Avena Coleoptile by Hydroxyproline," Plant Physiology, XLII (1967), 271-274.

[11] M. R. Stetten, "Mechanisms of Conversion of Ornithine into Proline and Glutamic Acid," Journal of Biological Chemistry, CLXXXIX (1951), 499.

[12] M. R. Stetten and R. Schoenheimer, "The Metabolism of L-Proline Studied with the Aid of Deuterium and Isotopic Nitrogen," Journal of Biological Chemistry, CLIII (1964), 113.

[13] R. Cleland, "Inhibition of Cell Elongation in Avena Coleoptile by Hydroxyproline," Plant Physiology, XLII (1967), 271-274.

Evidence of an arginine reversal influence in IAA was not observed in this study, but 15% reversal was obtained in water.

It was noted in this study that ATP reversed hydroxyproline inhibition and stimulated growth in the absence of hydroxyproline. Complete reversal was obtained in water, and partial reversal was noted in IAA. Norris obtained similar results with ethionine.[14] Cantoni's studies have shown that methionine can be activated by ATP to form S-adenosylmethionine and that ethionine, an analogue of methionine, can combine with ATP to form S-adenosylethionine.[15] Unlike S-adenosylmethionine, S-adenosylethionine exerts an ATP-trapping effect since the adenosine moiety is not readily released from this compound.[16] Since ATP exhibits the ability to reverse ethionine inhibition, Norris has suggested that ethionine

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[14] W. E. Norris, Jr., "Reversal of Ethionine-Induced Inhibition of Elongation of *Avena* Coleoptiles by Adenosine Triphosphate," *Archives of Biochemistry and Biophysics*, CVII (1964), 352-355.

[15] G. L. Cantoni, "Activation of Methionine for Transmethylation," *Journal of Biological Chemistry*, CLXXXIX (1951), 745-749.

[16] S. Villa-Trevino, *et al.*, "The Role of Adenosine Triphosphate Deficiency in Ethionine-Induced Inhibition of Protein Synthesis," *Journal of Biological Chemistry*, CCXXXVIII (1963), 1757-1763.

inhibits protein synthesis of the Avena coleoptile by interfering with ATP metabolism.[17] There might be a correlation between the mechanism of hydroxyproline action and that of ethionine. Hydroxyproline, a structural analogue of proline, could possibly interfere with ATP metabolism by a mechanism similar to that of ethionine, but the effect of hydroxyproline on the cellular concentration of ATP must be studied before a precise comparison of the two mechanisms may be made.

The ATP may influence hydroxyproline inhibition indirectly. Glutamic acid and ATP exhibit similar effects on elongation in the presence or absence of hydroxyproline in water and in IAA. These results indicate a relationship between the reversal effects of ATP and glutamic acid. The conversion of glutamic acid into proline, which involves several steps requiring ATP and enzymes, has been reported by Stetten.[18] Addition of exogenous ATP might effectively increase the concentration of proline and reverse the inhibition produced by hydroxyproline.

It was noted in this study that ATP reduced hydroxyproline inhibition more in water than it did in IAA.

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[17] Norris, op. cit.

[18] M. R. Stetten, "Mechanisms of Conversion of Ornithine into Proline and Glutamic Acid," Journal of Biological Chemistry, CLXXXIX (1951), 499.

Marré and Forti reported that auxin caused a definite increase in the ATP level in pea internode segments.[19] Ormrod and Williams observed that other growth regulators, gibberellic acid and 2,4-D, increased the level of acid soluble organic phosphorus and decreased the inorganic phosphorus.[20] This increase of the ATP concentration may be a cause of the reduction of ATP reversal of hydroxyproline inhibition in IAA. No precise conclusion concerning the role that ATP plays in hydroxyproline inhibition can be drawn from this preliminary work.

Cleland reported that sucrose increased auxin-induced elongation and increased the amount of hydroxyproline inhibition in IAA.[21] Schneider noted that a sugar extract of the endosperm of Avena seedlings promoted growth in the presence of auxin.[22] Results from this study indicate that

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[19] E. Marré and G. Forti, "Metabolic Responses to Auxin III. The Effects of Auxin on ATP Level as Related to the Auxin-Induced Respiration Increase," Physiologia Plantarum, XI (1958), 36-47.

[20] D. P. Ormrod and W. A. Williams, "Phosphorus Metabolism of Trifolium hirtum as Affected by 2,4-Dichlorophenoxyacetic Acid and Gibberellic Acid," Plant Physiology, XXXV (1960), 81-87.

[21] R. Cleland, "Inhibition of Cell Elongation in Avena Coleoptile by Hydroxyproline," Plant Physiology, XLII (1967), 271-274.

[22] C. L. Schneider, "The Interdependence of Auxin and Sugar for Growth," American Journal of Botany, XXV (1938), 258-270.

sucrose increases the auxin-induced elongation and the degree of hydroxyproline inhibition. Schneider found that the effects of auxin and sugar on growth are interdependent.[23] An increase in concentration of either auxin or sugar yields an increase in growth. This might explain the increased elongation when sucrose is added to IAA. Sucrose did not affect hydroxyproline inhibition in water, but a large increase was detected in the growth-stimulating effect of 2.5×10^{-4}M and 5×10^{-4}M ATP. Sucrose had no influence on the ability of ATP to reduce inhibition in water or in IAA. The mechanism of sucrose action upon hydroxyproline inhibition remains unclear.

A surprising result of this study was the observation that guanine completely reversed hydroxyproline inhibition in water. No significant decrease in the inhibition was noted in IAA. Elongation in 2.5×10^{-4}M guanine was twice as much as in water. Only 10% stimulation was noted in IAA. The following postulation might explain the mode of guanine action. Data presented in this paper have shown that glutamic acid reverses hydroxyproline inhibition of Avena coleoptile elongation. Since Abrams and Bentley have reported that glutamic acid is involved in the conversion

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[23] Ibid.

of inosinic acid into guanylic acid,[24] the addition of exogenous guanine might increase the glutamic acid concentration by a feedback mechanism and reduce hydroxyproline inhibition.

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[24] R. Abrams and M. Bentley, "Transformation of Inosinic Acid to Adenylic and Guanylic Acids in a Soluble Enzyme System," Journal of American Chemical Society, LXXVII (1955), 4179.

CHAPTER V

SUMMARY

The effect of hydroxyproline on Avena coleoptile elongation in water and in 3-indoleacetic acid (IAA) has been measured. Hydroxyproline at a concentration of 1×10^{-2}M is an effective inhibitor of Avena coleoptile elongation. Sucrose increases the degree of inhibition in IAA. Proline completely reverses the hydroxyproline inhibition of elongation in water and in IAA. Adenosine triphosphate (ATP), glutamic acid, and guanine completely reverse the hydroxyproline inhibitory effect in water and partially reverse the inhibition in IAA. Ornithine partially reverses the hydroxyproline inhibition in water and in IAA. The ability of ATP to reverse hydroxyproline inhibition is not altered markedly by the presence of sucrose.

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