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**COMPARATIVE EFFECTS OF GIBBERELIC ACID AND N(6)-  
BENZYLADENINE ON GROWTH AND DEVELOPMENT OF DWARF  
WATERMELON SEEDLINGS**

*University of New Hampshire*

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COMPARATIVE EFFECTS OF GIBBERELIC ACID AND  
N<sup>6</sup>-BENZYLADENINE ON GROWTH AND DEVELOPMENT  
OF DWARF WATERMELON SEEDLINGS

BY

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B.S. (Plant Science), University of Massachusetts, 1975

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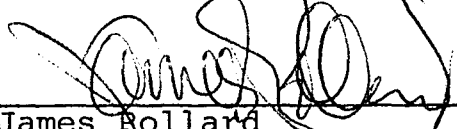
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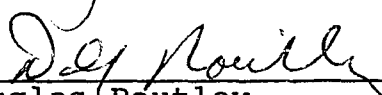
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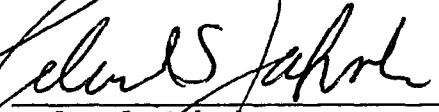


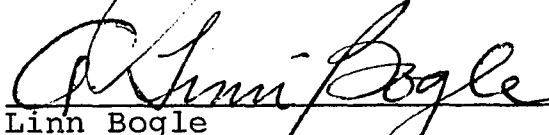
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ABSTRACT

COMPARATIVE EFFECTS OF GIBBERELLIC ACID AND  
N<sup>6</sup>-BENZYLADENINE ON GROWTH AND DEVELOPMENT  
OF DWARF WATERMELON SEEDLINGS

by

Cheryl D. Zack

University of New Hampshire, September 1981

Apical applications of 0.2 micrograms N<sup>6</sup>-benzyladenine (BA), a synthetic cytokinin, or 5 micrograms gibberellic acid (GA) significantly enhanced hypocotyl elongation in intact dwarf watermelon seedlings. Accompanying the increase in hypocotyl length was increased expansion of the cotyledons and inhibition of root growth. A study on dry matter partitioning indicated that both hormones caused a preferential mobilization of metabolites from the cotyledons to the hypocotyl at an expense to roots. However, in comparison to untreated or GA-treated seedlings, BA decreased total translocation of metabolites out of the cotyledons. Hormonal effects on water potential of cotyledons and hypocotyls were determined by allowing organs to equilibrate for 2 h in serial concentrations of polyethylene glycol 4000. Osmotic potentials were

determined with a dewpoint microvoltmeter. Results of a time-course study of hormonal effects on water and osmotic potentials indicated that BA stimulated cotyledon expansion and hypocotyl elongation primarily by increasing cell wall extensibility, whereas GA-promotion of cotyledon expansion and hypocotyl growth involved osmoregulation in addition to a lowering of the yielding threshold of the cell wall. Measurements of soluble sugars by a colorimetric procedure and cations by atomic absorption spectrometry confirmed that GA promoted the accumulation of osmotically active solutes in the cotyledons and hypocotyls.

## INTRODUCTION

Exogenous application of N<sup>6</sup>-benzyladenine (BA), a synthetic cytokinin, or gibberellic acid (GA) significantly enhances hypocotyl elongation in intact dwarf watermelon seedlings. BA-promotion of hypocotyl elongation is primarily a result of increased cell elongation (Loy, 1980) whereas, GA increases both cell size and cell number (Loy and Liu, 1974). Accompanying the BA-promoted increase in hypocotyl length is expansion of the cotyledons and inhibition of root growth. GA also inhibits root growth, but there have been no reported effects of GA on cotyledon expansion in dwarf watermelon seedlings.

Since both BA and GA affect cell elongation, it would be of interest to know if they affect this process through similar mechanisms. Cell enlargement requires a stretching of the existing cell wall plus synthesis of new wall material to maintain the properties of the cell wall for continued extension (Rayle, Haughton, and Cleland, 1970). The driving force for cell elongation is turgor pressure which exceeds some yielding threshold of the cell wall (Green, Erickson, and Buggy, 1971). The rate of cell elongation is controlled by turgor pressure and wall extensibility (Cleland, 1971). Actual cell enlargement is dependent on the uptake of water by the vacuole (Heyn, 1940). Water movement into a plant is regulated by the

osmotic potential of the vacuole and is limited by the rigidity of the cell wall. The rate of water movement into a cell can be accelerated by decreasing the osmotic potential of the cell or by wall loosening. Therefore, GA and BA must alter the growth potential of dwarf watermelon hypocotyls by either promoting the accumulation of osmotically active solutes within cells of the hypocotyl, by increasing cell wall extensibility, or by affecting both of the above phenomena.

The following objectives were set forth to compare the effects of GA and BA on seedling development in intact dwarf watermelon seedlings:

1. Determine a time-course of growth and biomass distribution in seedling organs.
2. Determine time-course changes in amounts and distribution of major solutes in seedling organs.
3. Determine changes in water and solute potentials over time in hypocotyls and cotyledons.

## LITERATURE REVIEW

### Cytokinin and GA Effects on Seedling Development

Exogenous application of cytokinins to intact plants usually results in suppression of stem elongation, inhibition of root growth, and promotion of cotyledon expansion (Banerji and Laloraya, 1967; Ikuma and Thimann, 1963; Metvier and Paulilo, 1980; Sprent, 1968; Wittwer and Dedolph, 1963). BA stimulated growth of excised watermelon cotyledons within 48 h following treatment (Longo et al., 1978a). High concentrations ( $10^{-4}$ M) promoted hypocotyl elongation in tomato seedlings (Aung and Byrn, 1976), and Loy (1980) reported that low dosages of BA significantly increased hypocotyl elongation in WB-2 dwarf watermelon seedlings, primarily through its effect on cell size. This increased rate of growth was detectable within 6 h following treatment and persisted for 48-60 h.

It has been repeatedly shown that exogenous application of GA promotes stem elongation in genetically dwarfed plants (Brian and Hemming, 1955; Cooper, 1958; Denna, 1963; Phinney, 1956) as well as in several normal plants (Wittwer and Bukovac, 1962; Wittwer and Dedolph, 1963). Accompanying GA-promoted increases in stem elongation is an inhibition of root growth (Garcia-Luis and Guardiola, 1978; McComb, 1966; Rai and Laloraya, 1967; Wittwer and Dedolph, 1963). GA

stimulates hypocotyl elongation and inhibits root growth of WB-2 dwarf watermelon seedlings (Loy and Liu, 1974). This GA promotion of watermelon hypocotyl elongation persists throughout 120 h following treatment, probably as a result of GA affecting both cell size and cell number. GA has either no effect on cotyledon expansion (Metvier and Paulilo, 1980) or causes a slight increase in cotyledon fresh weight (Letham, 1971; Rai and Laloraya, 1967).

#### Cytokinin and GA Effects on Osmotic Potential

The rate of breakdown of cotyledonary and, to some extent, hypocotyl reserves, and subsequent translocation and redistribution of these metabolites could change osmotic potentials within the seedling tissues. In watermelon cotyledons, the storage reserves are primarily lipids and proteins (Earle and Jones, 1962). During the first few days of seedling development, most of the loss in cotyledon dry weight is due to the breakdown of stored lipids and their subsequent translocation to other organs (Kagawa, McGregor, and Beevers, 1973). BA greatly accelerated lipid depletion in dark-grown, excised watermelon cotyledons after a lag period of 24 h (Longo et al., 1978a), and in dark-grown cucumber cotyledons following a 10 h lag period (Tsui et al., 1980). Accompanying the enhanced lipid breakdown was an increase in the amount of soluble sugars in both of these tissues. In dark-grown sunflower cotyledons (Servettaz et al., 1976) and watermelon cotyledons (Longo et al., 1978b)

the activity of glyoxylate cycle enzymes, involved in the conversion of lipids to soluble sugars, is enhanced by BA. In light-grown watermelon cotyledons, BA did not increase the activity of glyoxylate cycle enzymes and strongly accelerated their degradation (Lampugnani et al., 1980)

Cytokinins do not appear to lower the osmotic potential by increasing the amino acid pools. Huff and Ross (1975) reported no correlation between protein degradation to amino acids and zeatin-stimulated expansion of radish cotyledons. Cytokinins prevent protein loss in Avena stem segments (Jones and Kaufman, 1971a) and Xanthium leaves (Richmond and Lang, 1957). Cytokinins promote protein synthesis in pea stem segments (Banerji and Laloraya, 1967; Thimann and Laloraya, 1960) and inhibit proteolysis in bean cotyledons (Gilbert, Thompson, and Dunbroff, 1980), pea root tips (Shibaoka and Thimann, 1970), and corn leaves (Tavares and Kende, 1970). In contrast, cytokinins can replace the promotive action of the embryo axis on level of proteolytic activity in cotyledons of bean (Gepstein and Ilan, 1980) and squash seedlings (Penner and Ashton, 1966; 1967).

Although exogenously applied cytokinins have been reported to increase osmotically active solutes in cotyledons, cytokinins retard mobilization of reserves out of pea cotyledons (Banerji and Laloraya, 1967) and bean cotyledons (Gilbert, Thompson, and Dunbroff, 1980; Metvier and Paulilo, 1980). Within 24 h following treatment of intact dwarf watermelon seedlings with BA, there is a

preferential mobilization of substances from the cotyledons to the hypocotyl at an expense to root growth, but BA decreases total translocation of metabolites out of the cotyledons in comparison to non-treated seedlings (Loy, 1980).

Lipid degradation is also promoted by GA. Incubating barley aleurone layers in GA stimulated lipid breakdown with a lag period of 12 to 24 h (Firn and Kende, 1974). GA considerably enhanced the activity of isocitrate lyase, a key enzyme of the glyoxylate cycle, in endosperm of germinating castor beans (Marriot and Northcote, 1977; Wrigley and Lord, 1977) and in the cotyledons of dormant hazel seeds (Pinfield, 1968). The activity of catalase, a marker enzyme for glyoxsomal activity (Kagawa, McGregor, and Beevers, 1973), was enhanced by GA in light-incubated dwarf watermelon seeds (Evensen and Loy, 1978). GA also promotes invertase activity in some tissues (Broughton and McComb, 1971; Jones and Kaufman, 1971b; Kaufman et al., 1973). Increased rates of breakdown of sucrose associated with increased invertase activity could generate additional solutes within tissues. Kaufman, Ghosheh, and Ikuma (1968) reported that GA-promotion of growth and invertase activity in Avena internodes were closely parallel over the entire concentration range of GA used in their study. The kinetics of invertase activity was closely correlated to the kinetics of internode elongation in GA-treated pea seedlings (Broughton and McComb, 1971).



It seems unlikely that GA substantially increases the level of solutes by proteolysis. GA failed to restore the loss in proteolytic activity caused by removal of axial tissue in squash cotyledons (Penner and Ashton, 1966). GA had no effect on the rate of protein degradation in pea cotyledons (Garcia-Luis and Guardiola, 1978), and had little effect on amino acid pools during germination of dwarf watermelon seeds (Evensen and Loy, 1978). In contrast, Rai and Laloraya (1965) reported an increase in soluble-N content in GA-treated lettuce seedlings. Protein synthesis is necessary for GA-induced elongation of pea internodes (Broughton, 1969) and for GA-promoted growth and invertase activity in developing Avena internodes (Kaufman, Ghosesh, and Ikuma, 1968).

In contrast with cytokinins, GA does not appear to retard the mobilization of reserves out of cotyledons (McComb, 1966; Metvier and Paulilo, 1980). GA enhanced the mobilization of nitrogen reserves from the cotyledon to the hypocotyl of lettuce seedlings (Rai and Laloraya, 1965).

#### Cytokinin and GA Effects on Cell Wall Extensibility

An increase in cell wall extensibility could promote cell elongation by lowering the yielding threshold of the cell wall and allowing a greater movement of water into the plant. Longo et al. (1978a) have concluded that BA enhanced expansion of excised watermelon cotyledons by directly influencing the cell wall extensibility since the

osmotic potential of BA-treated cotyledons was always more positive than that of control cotyledons. An increase in cell wall extensibility may be invoked in BA promotion of hypocotyl elongation in dwarf watermelon seedlings as suggested by their flaccid appearance in comparison to untreated seedlings (Loy--personal communication). Marre et al. (1974) have shown that BA-promoted expansion of squash and radish cotyledons is correlated with an increase in the rate of proton extrusion. These results are in accordance with the acid growth theory, which has been proposed to explain auxin induced cell wall loosening (Rayle and Cleland, 1970).

GA has been shown to increase wall extensibility in Avena stem segments (Adams et al., 1975), pea stem segments (Lockhart, 1960), and lettuce hypocotyl sections (Stuart and Jones, 1977). However, GA had no effect on cucumber hypocotyl extensibility (Cleland et al., 1968; Katsumi and Kazama, 1978). The mechanism of GA-induced cell wall loosening is not known. GA had little effect on cellulase and pectinase activities in pea internodes (Broughton and McComb, 1971). Stuart and Jones (1978a) reported that GA-promotion of cell extensibility in lettuce hypocotyl sections is not explained by the acid-growth hypothesis since the kinetics and magnitude of GA- and acid-induced growth responses differ. However, Hebard et al. (1976) have shown that the presence of GA causes acidification in Avena stem segments.

## MATERIALS AND METHODS

### Plant Material

A dwarf inbred strain of watermelon (Citrullus lanatus (Thunb.) Matsu and Nakai), designated WB-2, was used in this study. Mechanically scarified seeds were germinated in the dark in a growth chamber at 29±1 C. After 72 h, seedlings were transferred to continuous light with a photon flux density of  $4.2 \mu\text{E m}^{-2} \text{s}^{-1}$  (400-700 nm) produced from 4 GTE cool white fluorescent lamps. After 96 h, the seed coats were removed and the seedlings were transferred to plastic Petri dishes (25 mm X 100 mm) containing absorbent wadding discs saturated with distilled water. Ten seedlings were placed in each dish with radicles inserted into absorbent wadding to prevent root desiccation. Seedlings were selected for hypocotyl uniformity and treated after 120 h.

### Hormone Treatments

Either 0.2  $\mu\text{g}$  BA or 5  $\mu\text{g}$  GA in a 10  $\mu\text{l}$  aqueous droplet was administered with a microsyringe to the apex of the seedlings between the cotyledons. These dosages elicited optimal hypocotyl elongation.

### Fresh and Dry Weight Determinations

Fresh and dry weight measurements of the cotyledon, hypocotyl, and root were taken at 6 h intervals for the first 48 h following treatment. Because of the large number of seedlings involved, GA and BA experiments were conducted separately, each with its own set of control seedlings. Four seedlings per replication and 3 replications were used for each determination.

### Respiration Rate Determination

Oxygen consumption of excised cotyledons was determined using a Gilson differential respirometer. The cotyledons were placed in a Warburg vessel in the dark at 29° C. Each treatment was replicated 3 times with 3 cotyledon pairs per replication.

### Water and Osmotic Potential Determinations

Water and osmotic potentials of cotyledons and hypocotyls were determined at 0, 12, 24, and 48 h following treatment. Two organs per replication and 5 replications were used for each determination. The cotyledons and hypocotyls were excised from intact seedlings at the time of measurement. Water potential was determined by placing the cotyledons or hypocotyls in serial concentrations of polyethylene glycol 4000 (PEG) of known osmotic potential. The osmotic potential of the solution that did not induce a measurable change in fresh weight of the tissue after 2 h

was assumed to equal the water potential of the organ. Osmotic potentials were determined by saturating filter paper discs with cell sap from homogenized cotyledons or hypocotyls. Discs were immediately placed in a Wescor C-52 chamber connected to a dewpoint microvoltmeter (Wescor HR-33T) which was calibrated against NaCl solutions.

#### Analysis of $K^+$ , $Ca^{2+}$ , and $Mg^{2+}$

$K^+$ ,  $Ca^{2+}$ , and  $Mg^{2+}$  levels were determined according to the method of Stuart and Jones (1977). Two cotyledons, hypocotyls, or roots were weighed and placed in small plastic vials in 1 ml of distilled deionized water and frozen. The tissue was thawed, 4 ml of water was added, and the tissue was homogenized and filtered. The filtrate was analyzed for  $K^+$ ,  $Mg^{2+}$ , and  $Ca^{2+}$  by atomic absorption spectrometry (Instrumentation Laboratory, Model 251). Each treatment was replicated 4 times.

#### Soluble Sugar Determinations

Cotyledons, hypocotyls, and roots were dried and powdered in a mortar. The dry powder was immersed in 80% ethanol overnight and extracted on a water bath at 60°C for 15 min. It was then filtered through a fine sintered glass funnel and the residue extracted with 60°C ethanol 3 times. The filtrates were combined and made up to a final volume. The filtrate was used for the determination of reducing sugars and total sugars according to the method of Cronin

and Smith (1979). Sucrose was determined as the difference between total sugars and reducing sugars. Each treatment was replicated 4 times with 5 organs per replication.

## RESULTS

### Hypocotyl Elongation

The time-course of hypocotyl elongation in BA- and GA-treated seedlings is shown in Fig. 1. Both hormones increased the rate of hypocotyl elongation in intact dwarf watermelon seedlings, but the maximum growth rate was elicited by GA. GA was most promotive between 24 to 72 h following treatment, whereas the maximum growth rate with BA occurred between 24 to 60 h.

### Seedling Development

Both GA and BA promoted cotyledon expansion, increased hypocotyl fresh weight, and inhibited root growth of intact seedlings (Fig. 2,3,4). Within 6 h following BA or GA treatment, cotyledon expansion was observed. Whereas cotyledons of BA-treated seedlings continued to expand for up to 48 h, GA-promotion of cotyledon expansion was slight and limited to the first 6 h. Cotyledon fresh weight of untreated seedlings remained nearly constant during the 48 h measurement period. The kinetics of changes in hypocotyl fresh weight and the kinetics of hypocotyl elongation were highly correlated ( $R^2=0.99$ ) for control, BA-, and GA-treated seedlings, thus indicating only slight, if any, promotion of lateral expansion of the hypocotyls by BA or GA treatments.

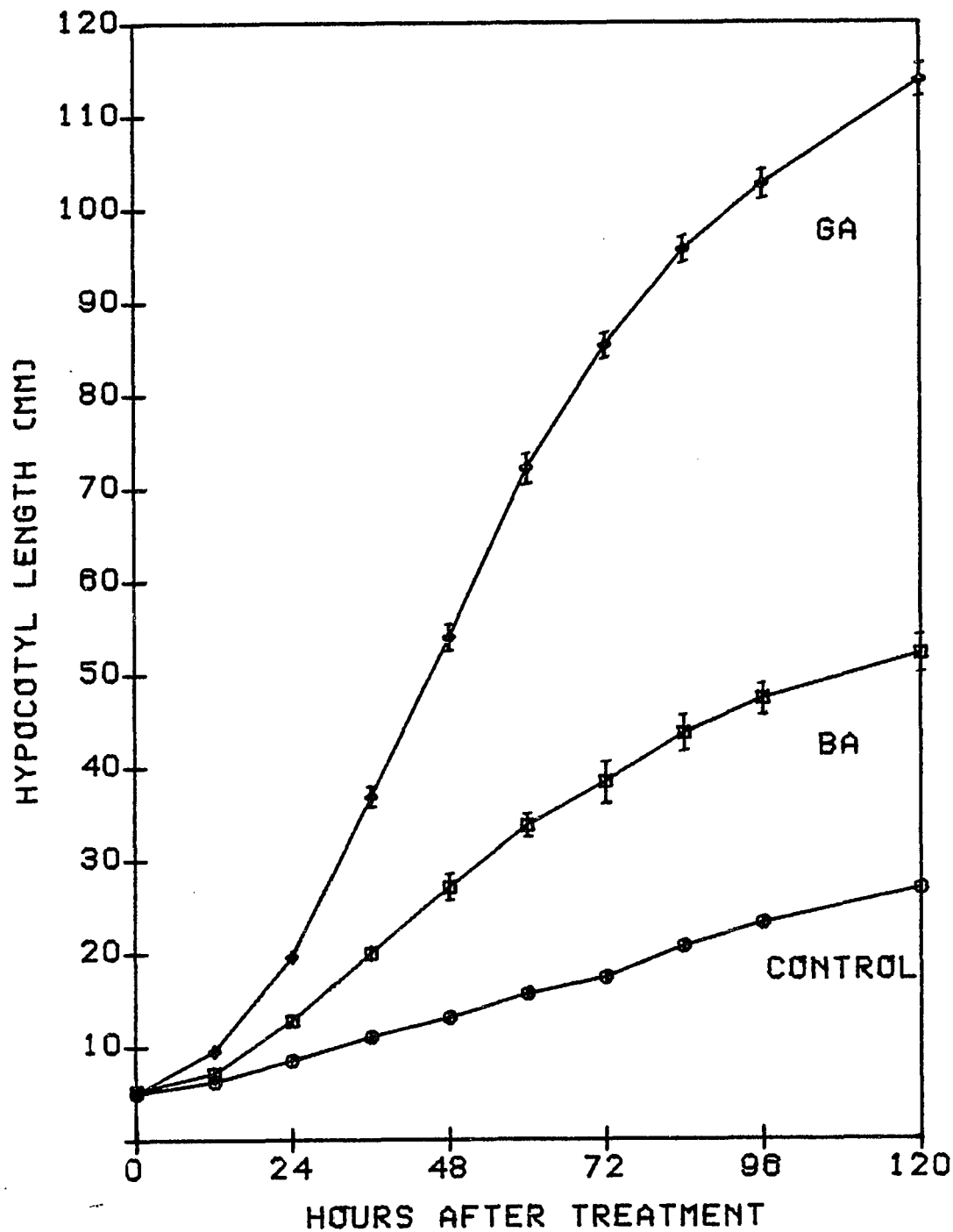


Fig. 1. Hypocotyl length of intact dwarf watermelon seedlings treated apically with 0.2  $\mu$ g BA or 5.0  $\mu$ g GA. Each point represents the average of 3 replications. Vertical bars indicate standard errors.



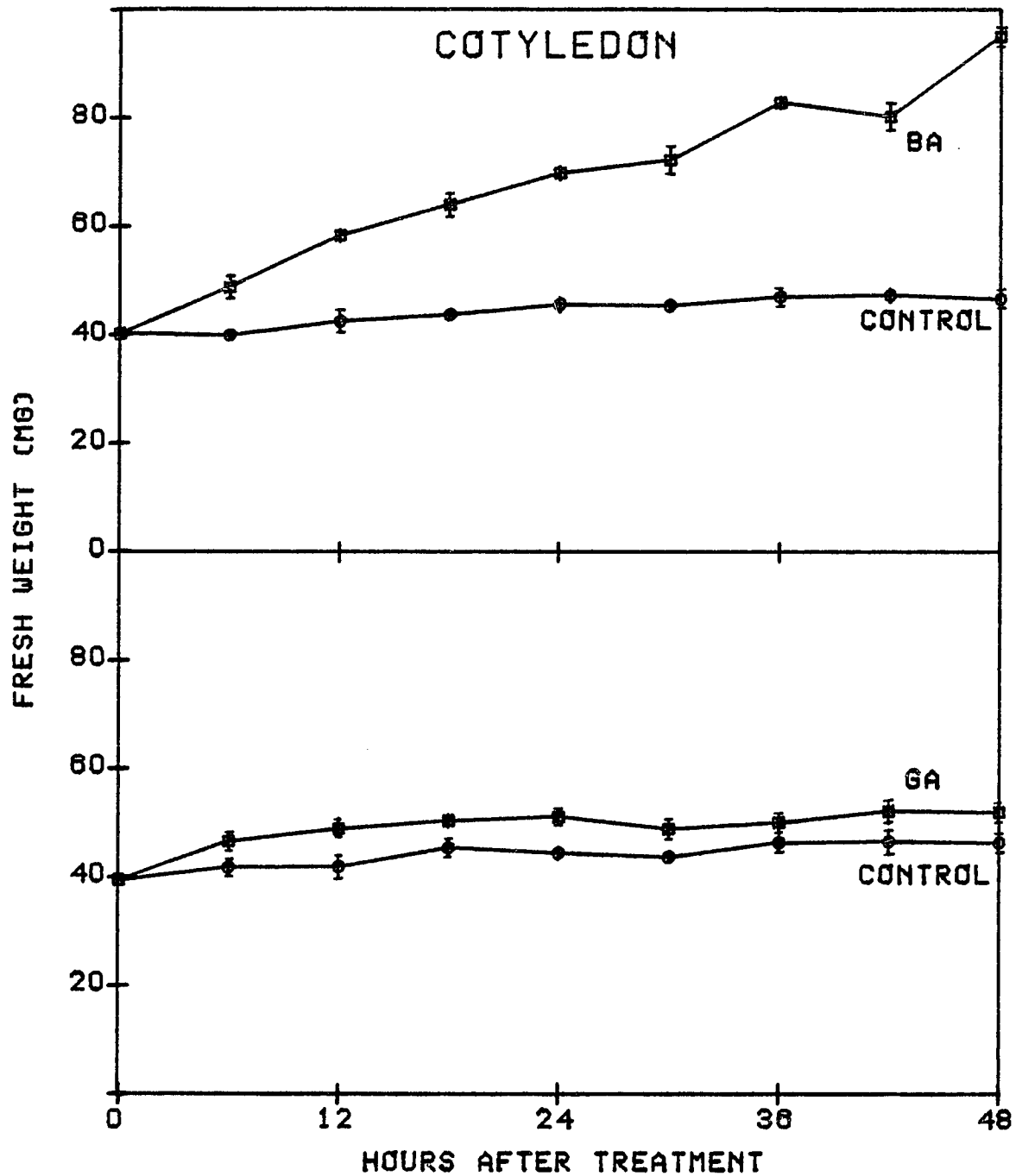


Fig. 2. Effect of BA and GA on fresh weight of intact dwarf watermelon cotyledons. Each point represents the average of 3 replications. Vertical bars indicate standard errors.

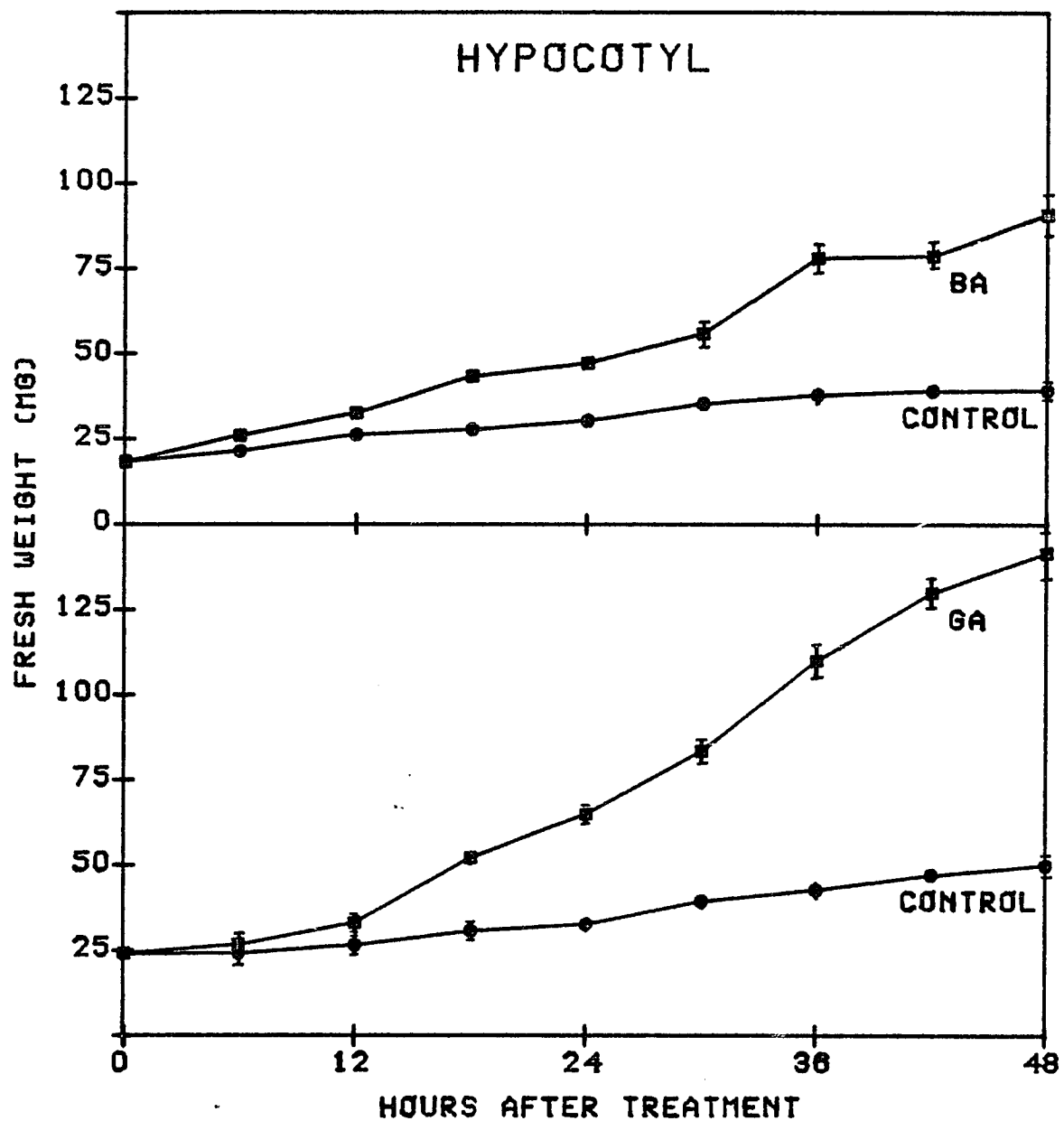


Fig. 3. Effect of BA and GA on fresh weight of intact dwarf watermelon hypocotyls. Each point represents the average of 3 replications. Vertical bars indicate standard errors.

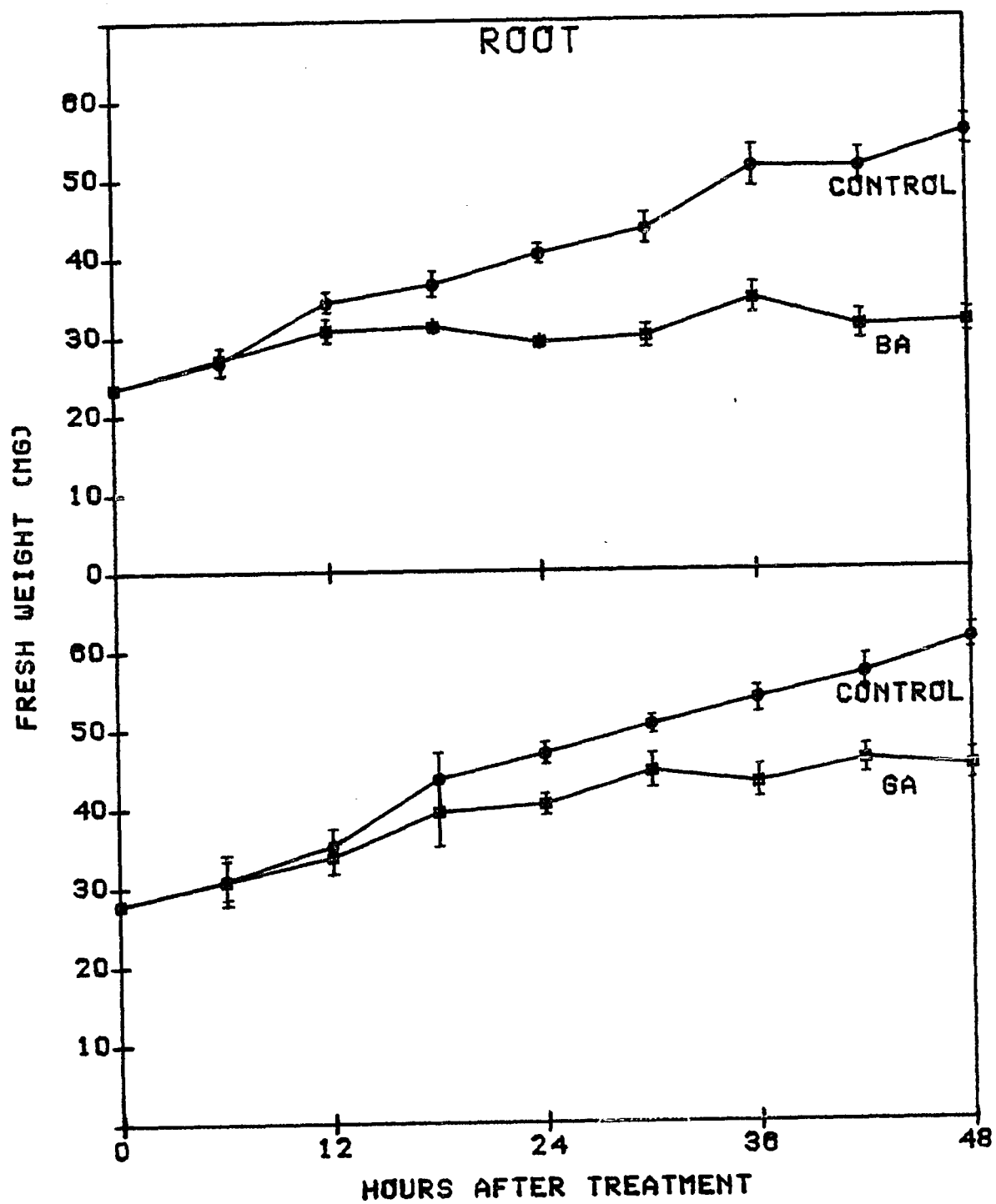


Fig. 4. Effect of BA and GA on fresh weight of intact dwarf watermelon roots. Each point represents the average of 3 replications. Vertical bars indicate standard errors.

Both BA and GA suppressed total root growth over 48 h, however, BA-suppression was most pronounced.

#### Dry Matter Partitioning

Both BA and GA caused a preferential mobilization of metabolites from the cotyledons to the hypocotyl at an expense to the roots (Fig. 5,6,7). However, in comparison to untreated or GA-treated seedlings, BA decreased total translocation of metabolites out of the cotyledons. Maximum accumulation of dry matter in hypocotyls was obtained with GA treatment. The GA-promoted increase in hypocotyl dry weight was detected by 12 h. A significant promotive effect of BA on hypocotyl dry weight was not detectable until 30 to 36 h following treatment. The kinetics of changes in root fresh and dry weights were closely parallel in all treatments.

#### Respiration Rate

The effect of BA and GA on respiration of excised cotyledons is shown in Table 1. Respiration, expressed as  $\mu\text{l O}_2$  absorbed per min per cotyledon pair, declined over the 48 h time period. BA enhanced respiration at all time periods, whereas, GA had no significant effect.

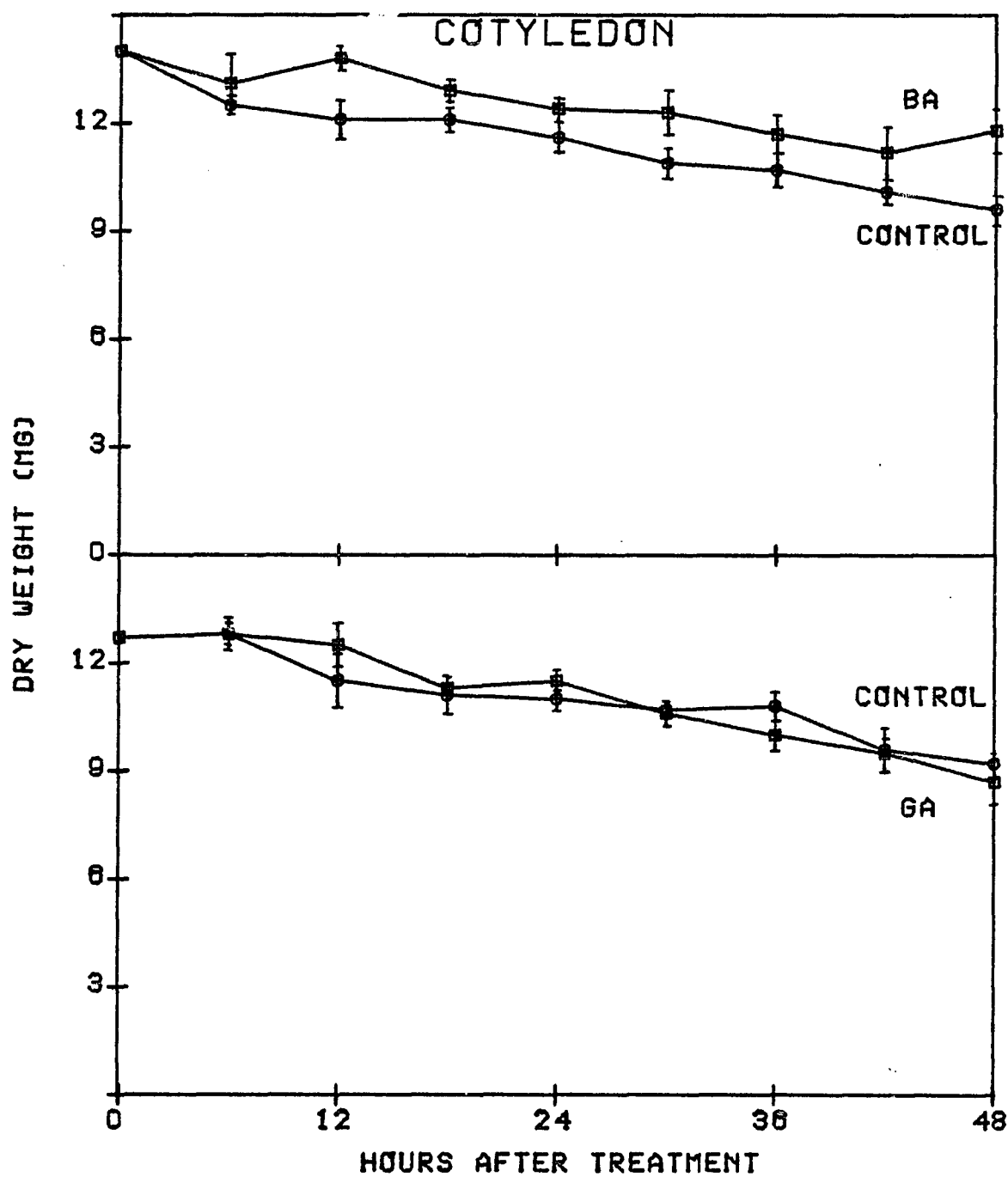


Fig. 5. Effect of BA and GA on dry weight of intact dwarf watermelon cotyledons. Each point represents the average of 3 replications. Vertical bars indicate standard errors.

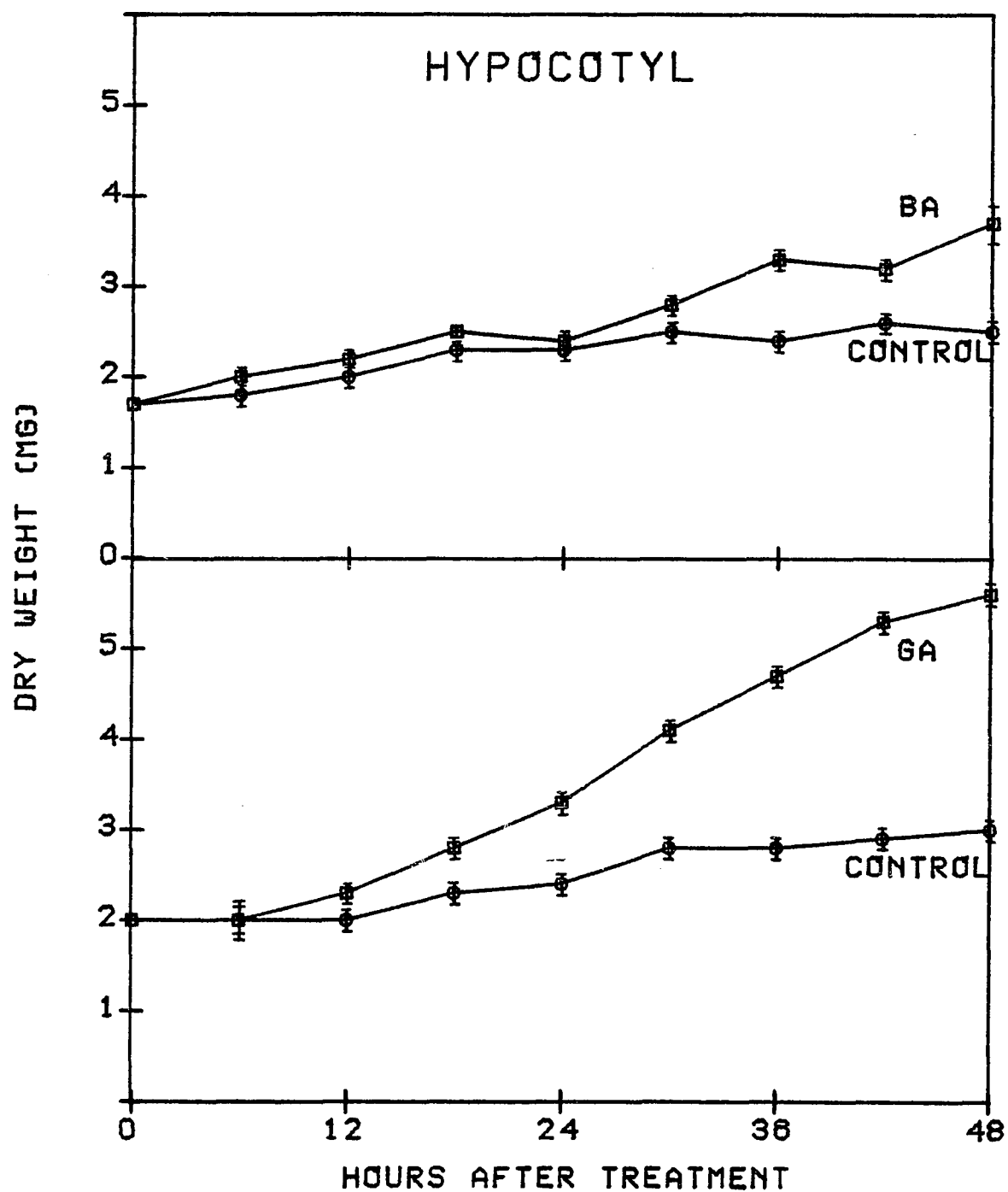


Fig. 6. Effect of BA and GA on dry weight of intact dwarf watermelon hypocotyls. Each point represents the average of 3 replications. Vertical bars indicate standard errors.

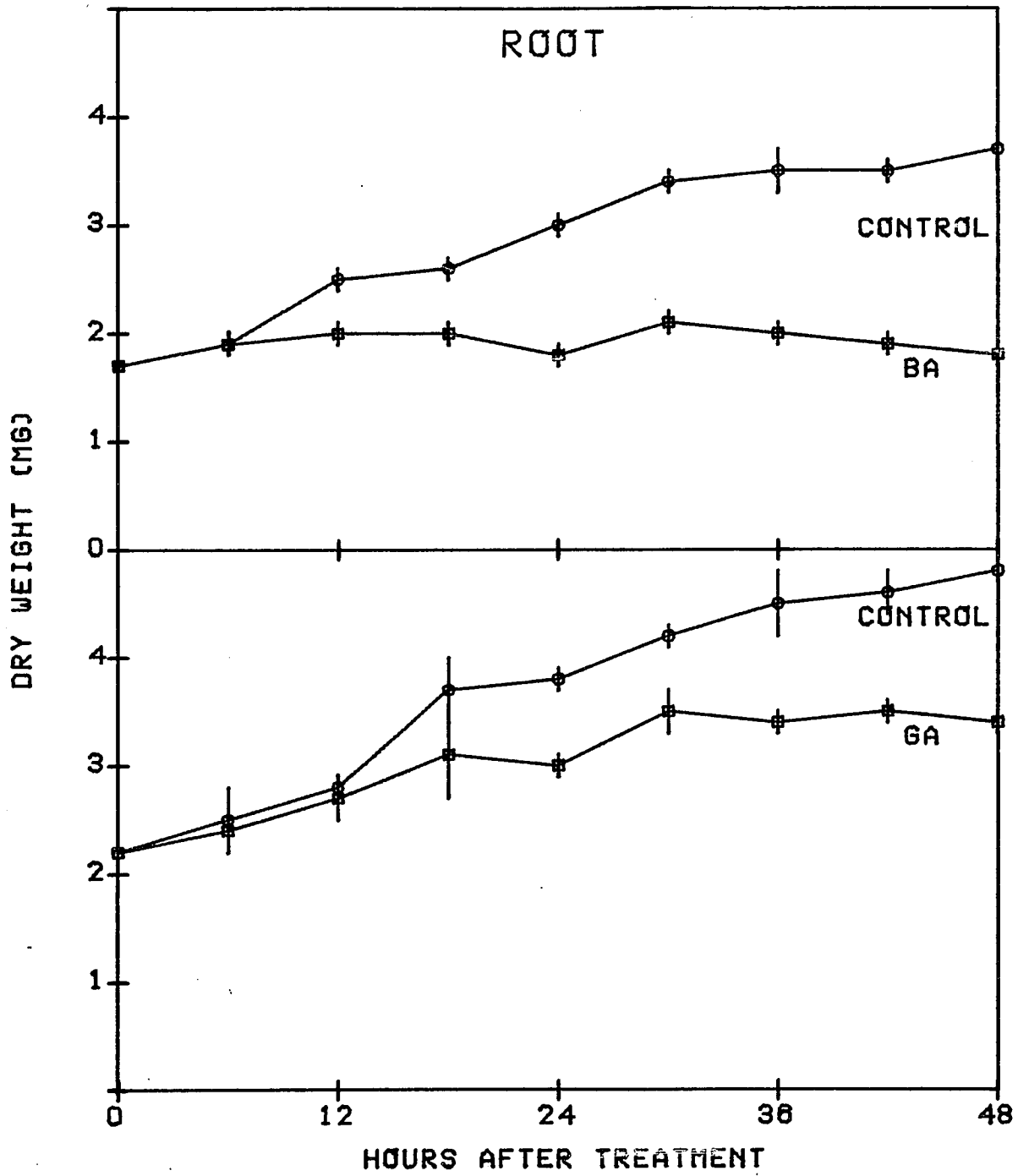


Table 1. Effect of 0.2  $\mu\text{g}$  BA or 5  $\mu\text{g}$  GA on respiration rate of excised watermelon cotyledons.

Hours Following Treatment	Treatment	$\mu\text{l O}_2$ Absorbed/Cotyledon pair/min <sup>1</sup>
0	Control	1.92a
24	Control	1.10b
	GA	1.27b
	BA	1.67a
48	Control	0.53d
	GA	0.67cd
	BA	0.99bc

<sup>1</sup>Mean separation (3 replicates) within column according to Duncan's Multiple Range Test, P = 0.05.



### Water and Osmotic Potentials of Intact Cotyledons

The effect of BA on water and osmotic potentials of intact cotyledons is illustrated in Fig. 8. The water potential of control cotyledons increased slightly from 12 to 24 h and then remained constant for the next 24 h. The osmotic potential continually increased over 48 h with the most rapid increase between 0 and 24 h. The water potential of BA-treated cotyledons did not differ statistically from controls except at 12 h following treatment when it was more positive. The osmotic potential of BA-treated cotyledons was always more positive than in control cotyledons, and the magnitude of the difference was greatest within 12 h following treatment.

Water potentials of GA-treated cotyledons were more negative than control cotyledons with the exception of 48 h following treatment when the values coincided (Fig. 9). At no time did the osmotic potential of GA-treated cotyledons differ significantly from that of control seedlings.

### Water and Osmotic Potentials of Intact Hypocotyls

Water potentials of untreated seedlings remained constant while the osmotic potential increased gradually over 48 h (Fig. 10 and 11). BA had no significant effect on hypocotyl water potentials, however, the mean values obtained were always less negative in BA-treated seedlings than in untreated seedlings (Fig. 10). Within 12 h following BA treatment, there was a sharp increase in the

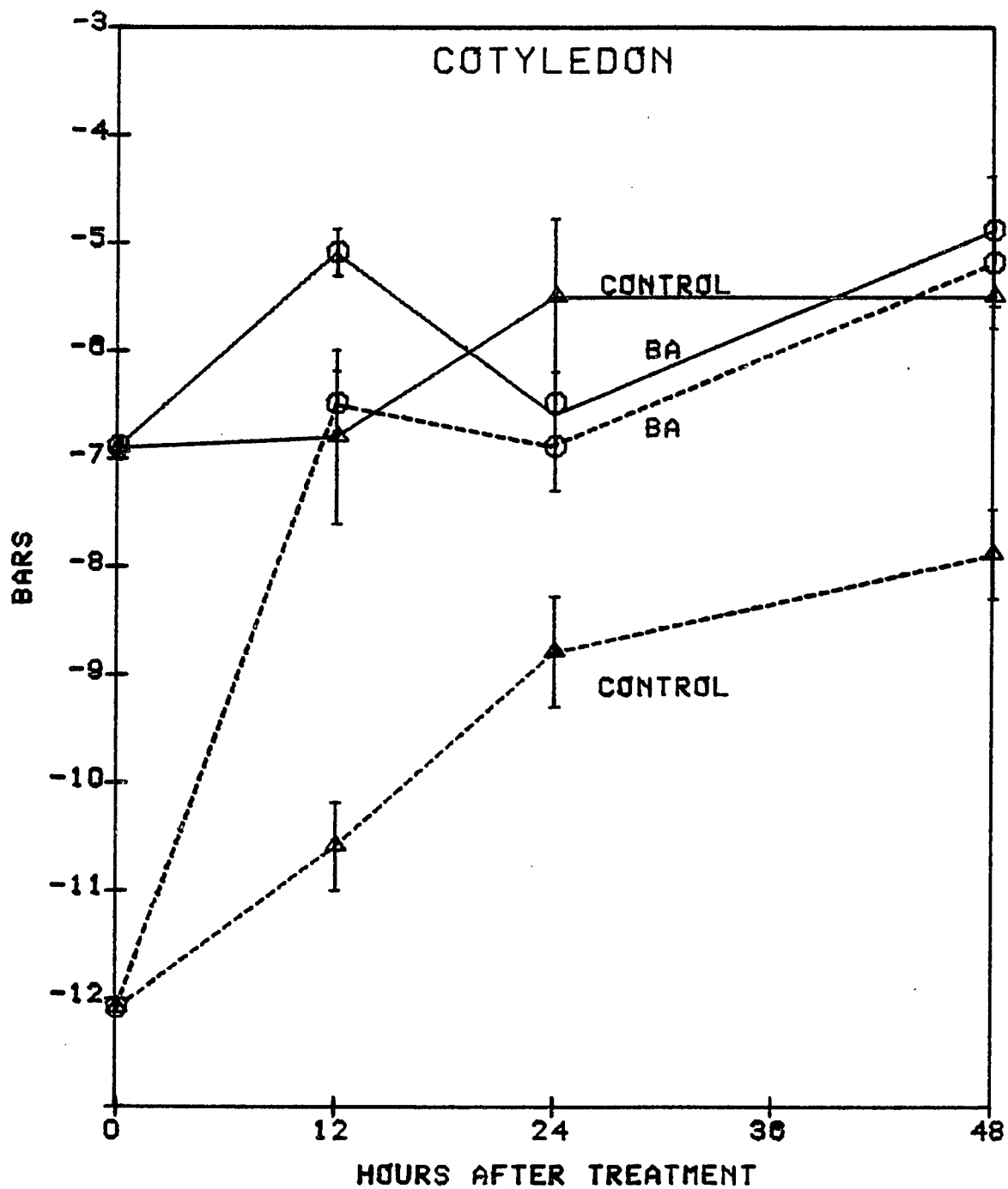


Fig. 8. Effect of BA on water potential (solid line) and osmotic potential (broken line) in intact cotyledons. Each point represents the average of 5 replications. Vertical bars indicate standard errors.

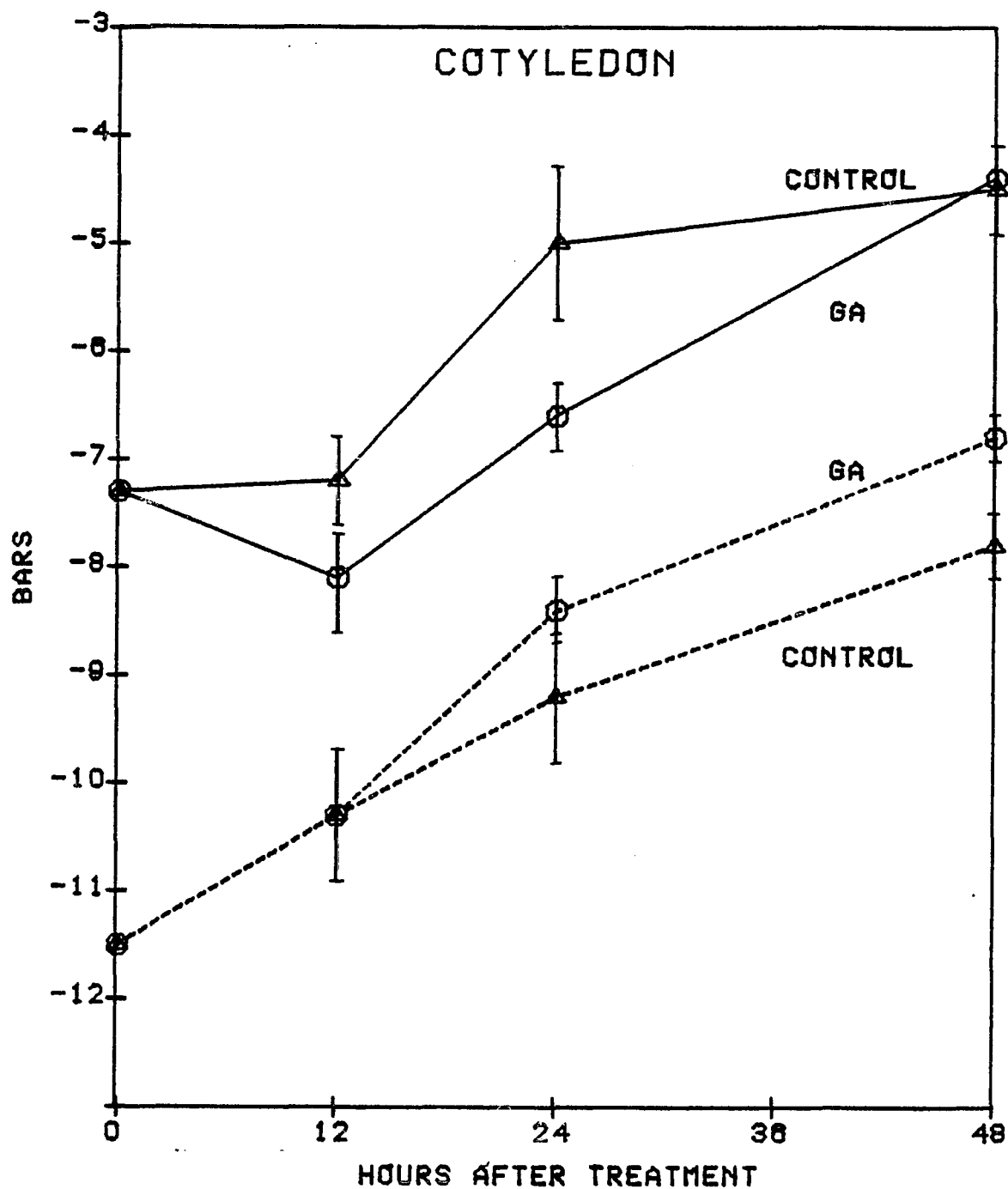


Fig. 9. Effect of GA on water potential (solid line) and osmotic potential (broken line) in intact cotyledons. Each point represents the average of 5 replications. Vertical bars indicate standard errors.

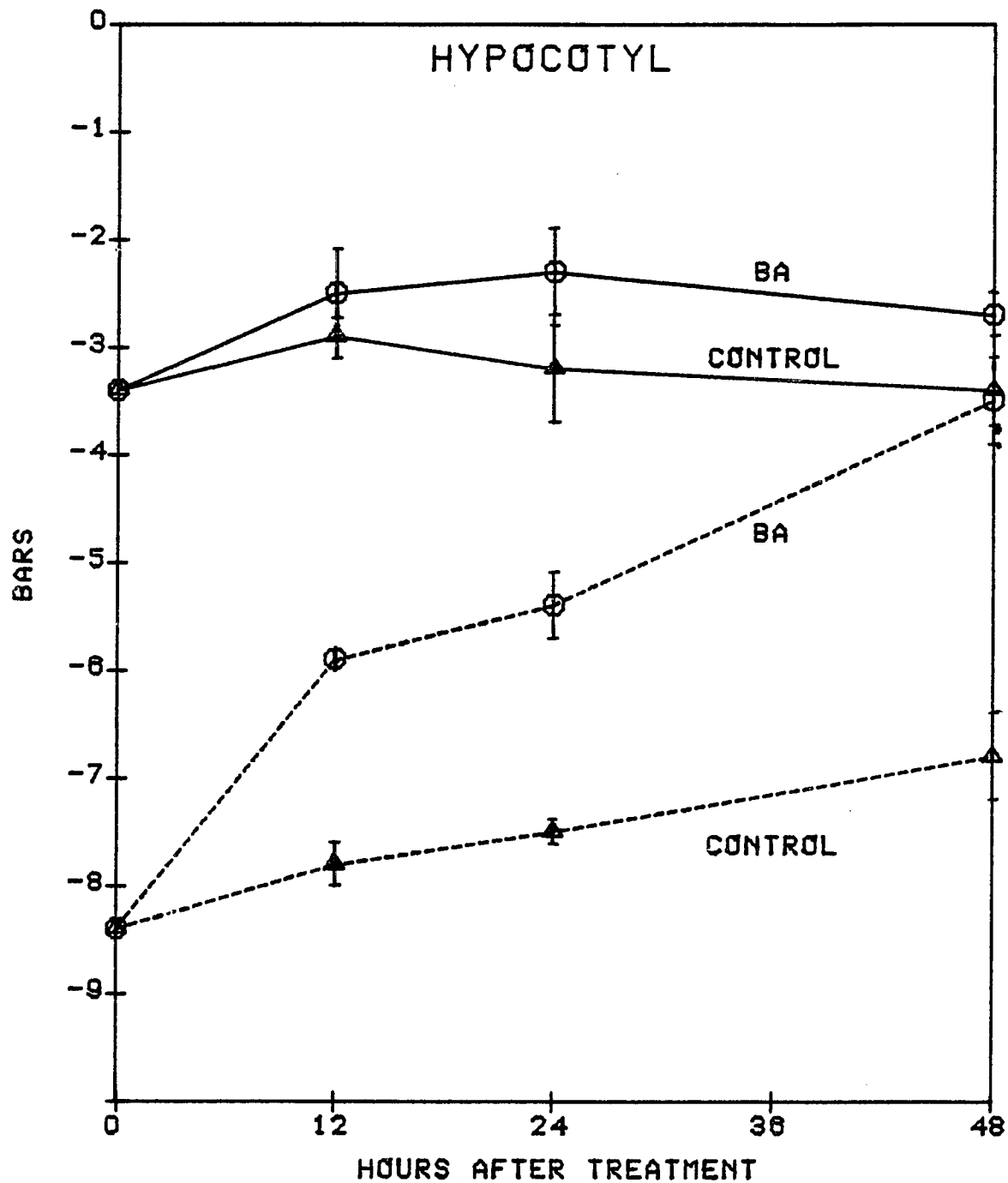


Fig. 10. Effect of BA on water potential (solid line) and osmotic potential (broken line) in intact hypocotyls. Each point represents the average of 5 replications. Vertical bars indicate standard errors.

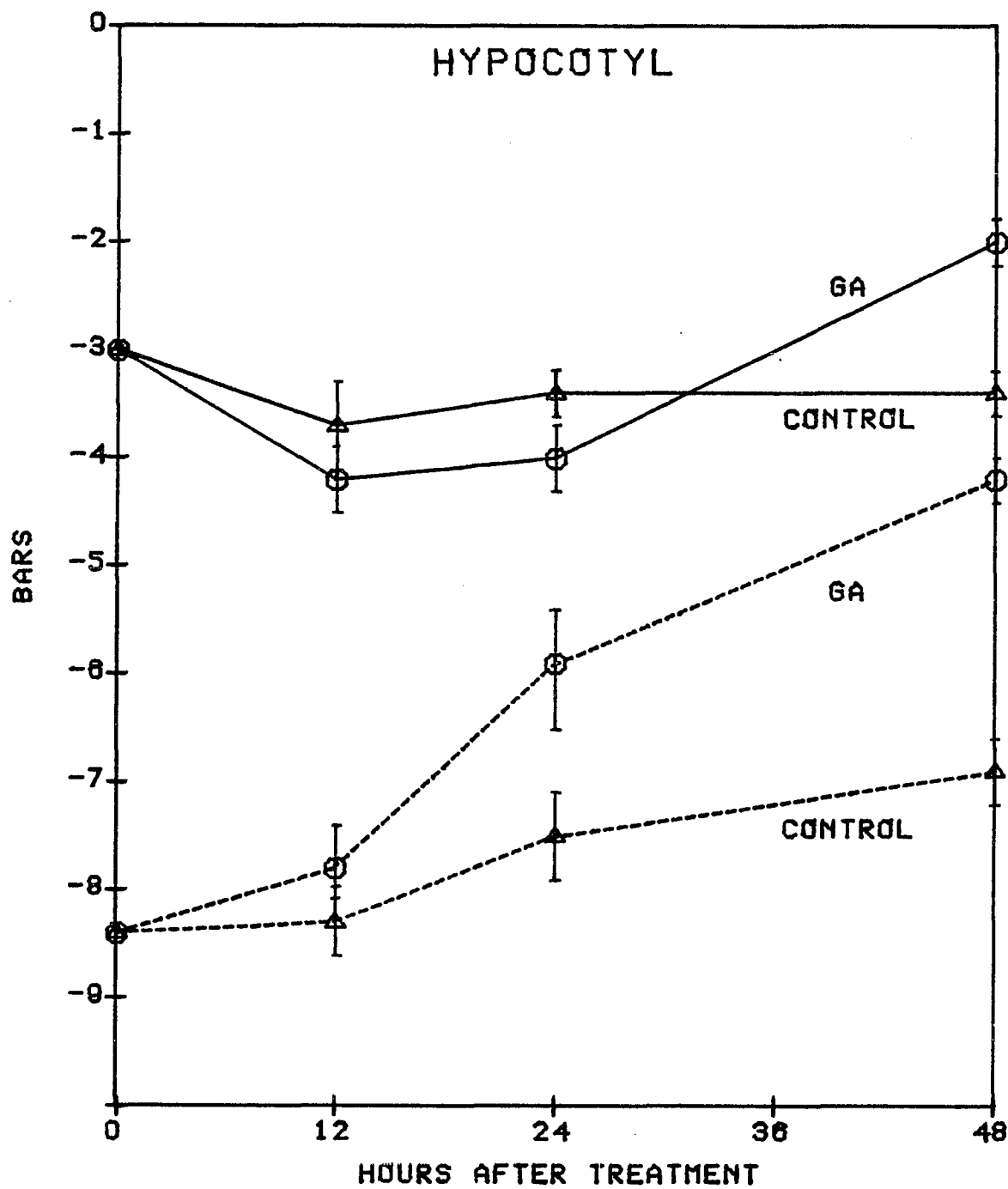


Fig. 11. Effect of GA on water potential (solid line) and osmotic potential (broken line) in intact hypocotyls. Each point represents the average of 5 replications. Vertical bars indicate standard errors.

osmotic potential, indicating that the concentration of solutes was not being maintained during BA-promoted growth. By 48 h, turgor pressure (calculated from differences between water potentials and osmotic potentials) of BA-treated hypocotyls was approaching 0 bars.

The osmotic potential of GA-treated hypocotyls increased at a faster rate than that of control hypocotyls, but there was a corresponding increase in water potential between 24 to 48 h so that turgor pressure was maintained during growth over that period.

### Solute Accumulation and Distribution

#### Reducing Sugars

Reducing sugar content of control cotyledons remained constant for 24 h and then sharply declined over the next 24 h period (Fig. 12). In GA-treated cotyledons, the level continually increased over the first 24 h and then decreased at a rate similar to that of untreated seedlings. In contrast with GA, BA had no significant effect during the first 24 h, and suppressed the decrease in reducing sugar content in the cotyledons during the second 24 h.

The pattern of reducing sugar accumulation in hypocotyls of intact seedlings differed significantly among treatments (Fig. 13). In control hypocotyls, the amount remained constant for 12 h, reached a maximum at 24 h, and then declined over the next 24 h period. In GA-treated hypocotyls, the amount of reducing sugars increased markedly

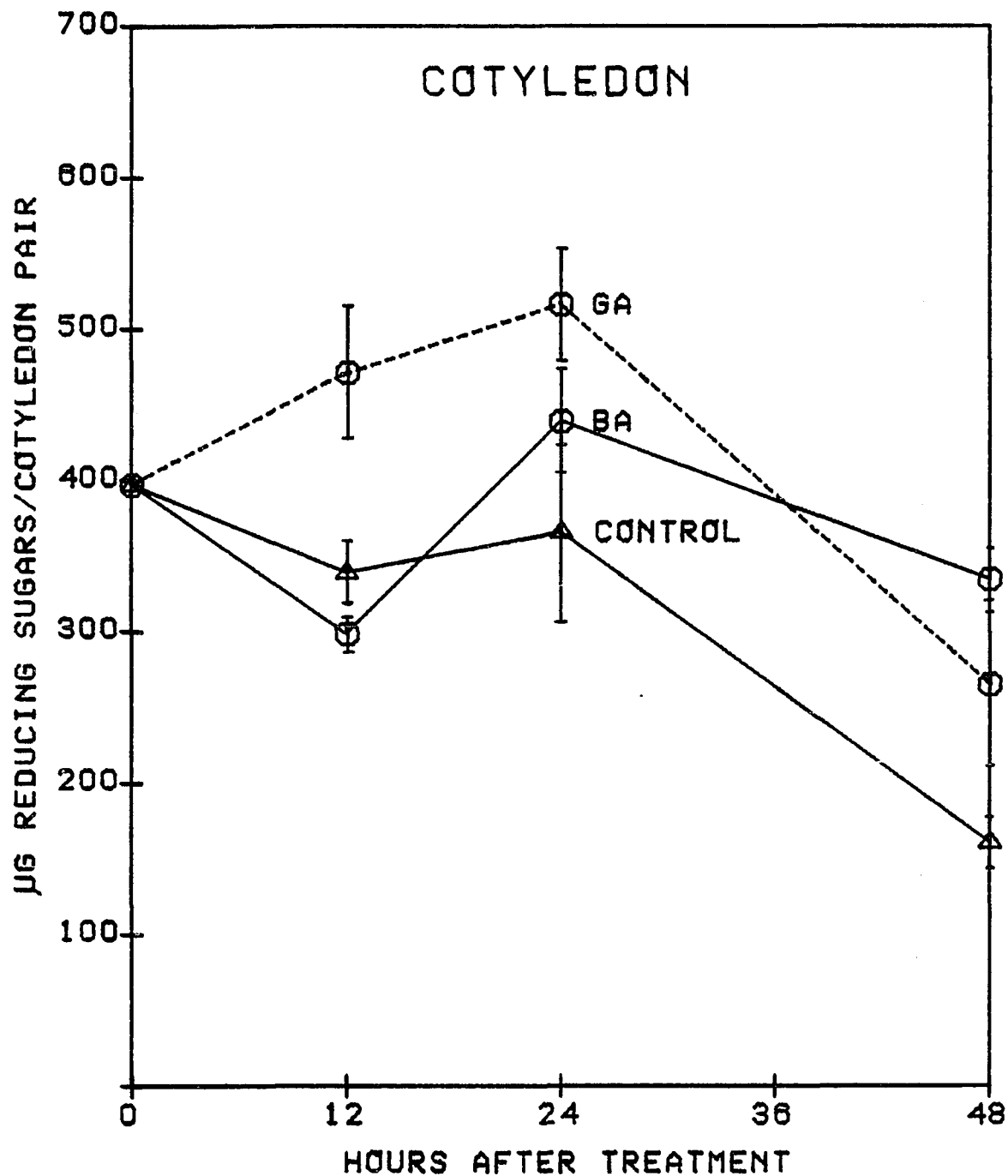


Fig. 12. Effect of BA and GA on reducing sugar content in intact watermelon cotyledons. Each point represents the average of 4 replications. Vertical bars indicate standard errors.

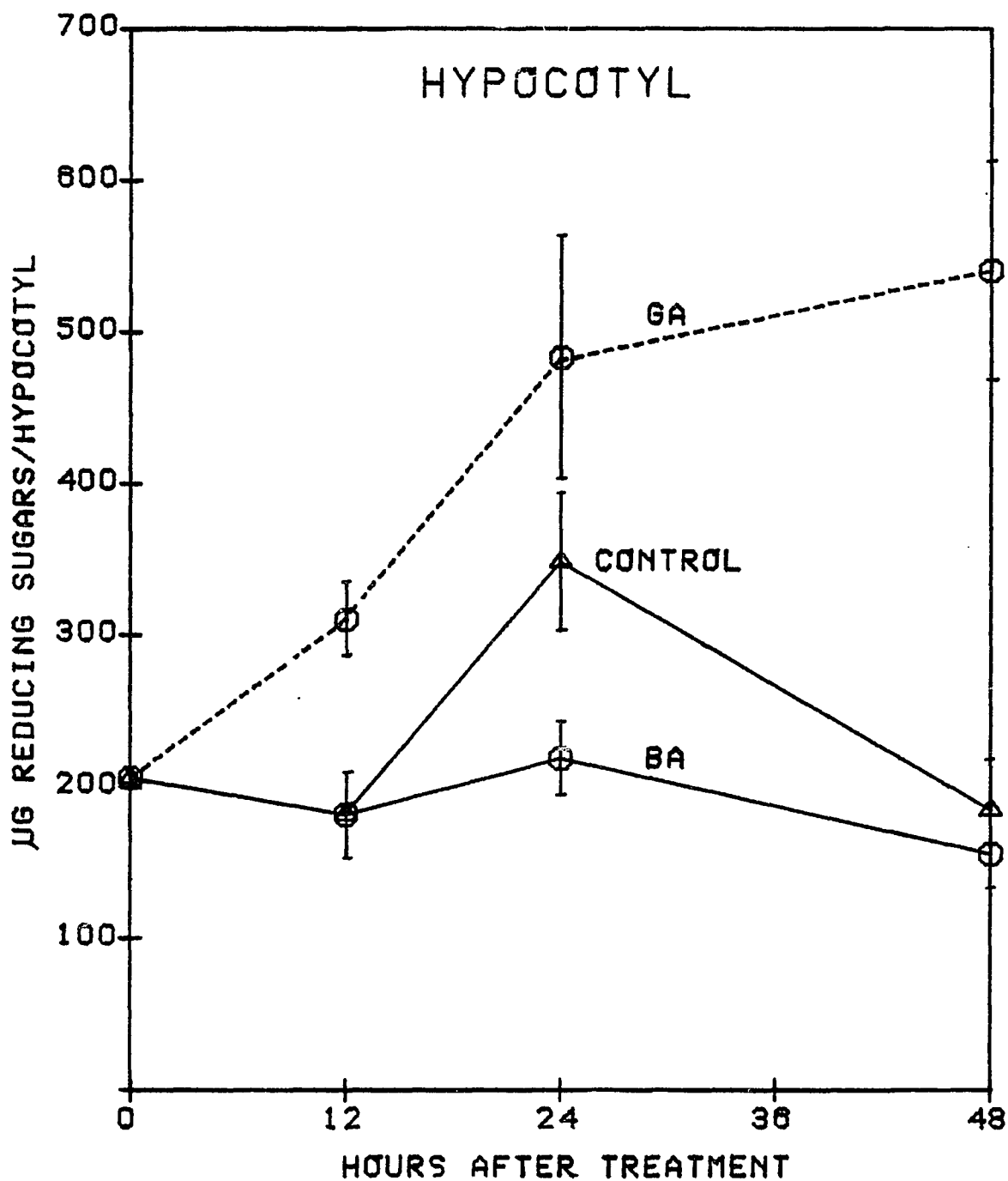


Fig. 13. Effect of BA and GA on reducing sugar content in intact hypocotyls. Each point represents the average of 4 replications. Vertical bars indicate standard errors.



within 12 h following treatment, continued to increase sharply until 24 h, and exhibited no significant change between 24 and 48 h. At 48 h following GA treatment, the amount of reducing sugars was at least 2.5 times as high as in the control hypocotyls. In BA-treated hypocotyls, reducing sugar levels did not change significantly over the 48 h period.

Amounts of reducing sugars in intact roots were quite variable within treatments as seedlings were selected on the basis of hypocotyl uniformity, but it is evident that both BA and GA inhibited the accumulation or maintenance of reducing sugars in intact roots (Fig. 14).

#### Non-reducing Sugars

In contrast with reducing sugars, non-reducing sugars (sucrose) levels were very low in intact seedling organs. During the first 12 h, sucrose levels reached a maximum in control and GA-treated cotyledons, and dropped almost 50% in BA-treated cotyledons (Fig. 15). During the following 36 h, the amount of sucrose in control cotyledons continually decreased. GA delayed this decrease for 12 h. At 48 h sucrose levels were similar in GA-treated and control cotyledons. In BA-treated cotyledons, sucrose levels increased from 12 to 24 h and then remained constant.

The amount of non-reducing sugars in intact hypocotyls was not significantly different in control or BA-treated seedlings over time; whereas, GA markedly enhanced levels after 12 h (Fig. 16). By 48 h following treatment, the

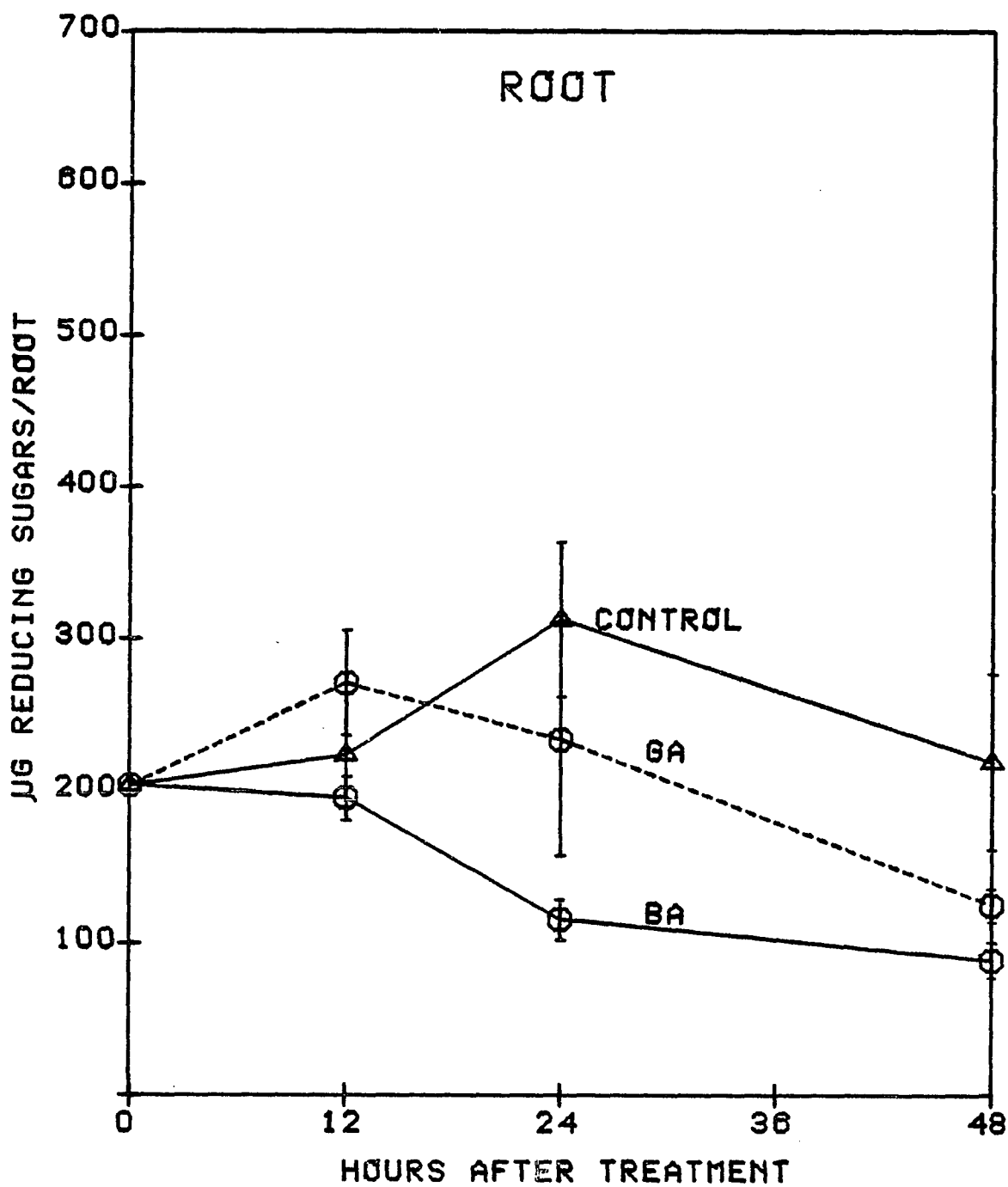


Fig. 14. Effect of BA and GA on reducing sugar content in intact roots. Each point represents the average of 4 replications. Vertical bars indicate standard errors.

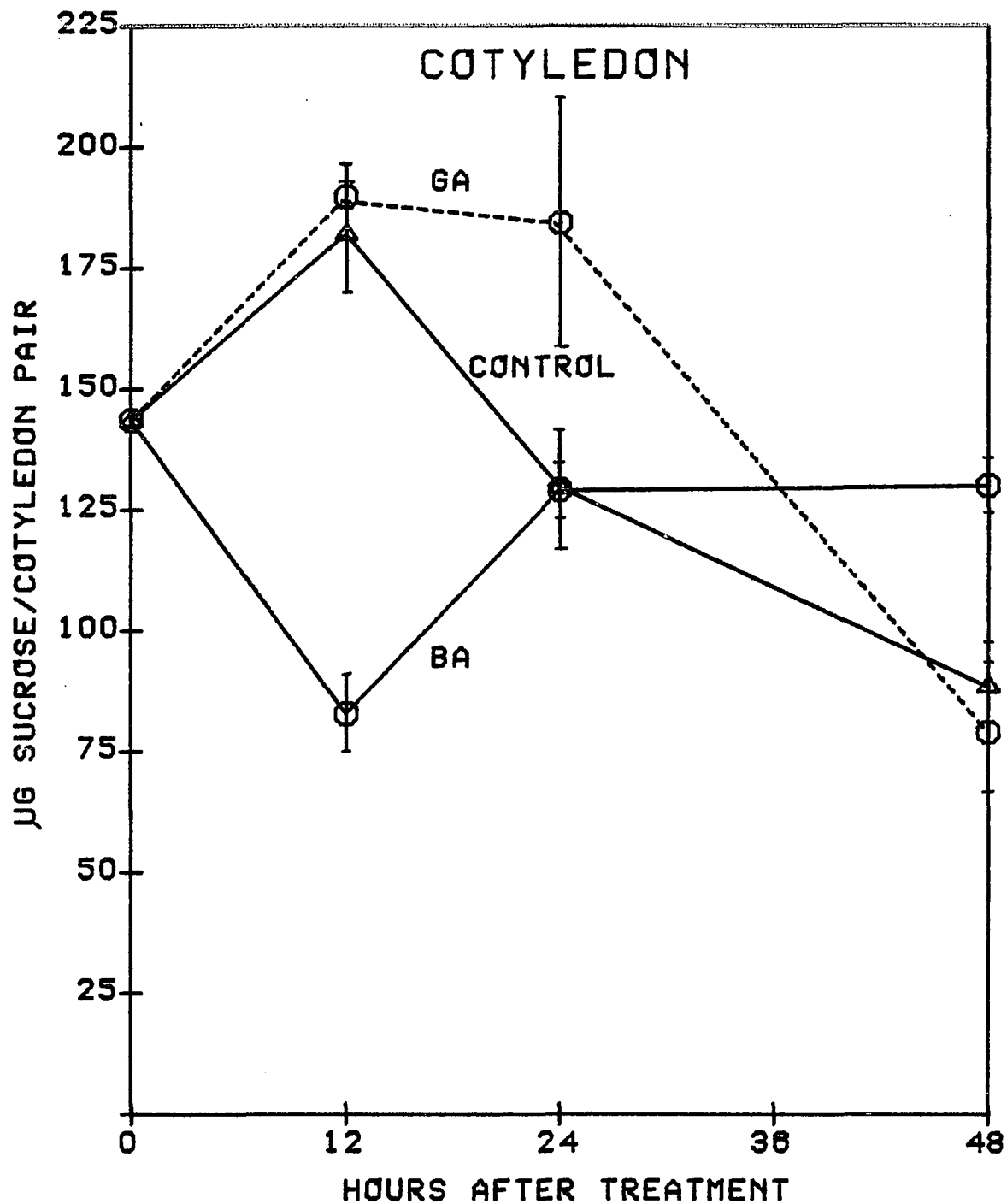


Fig. 15. Effect of BA and GA on sucrose (non-reducing sugars) content in intact cotyledons. Each point represents the average of 4 replications. Vertical bars indicate standard errors.

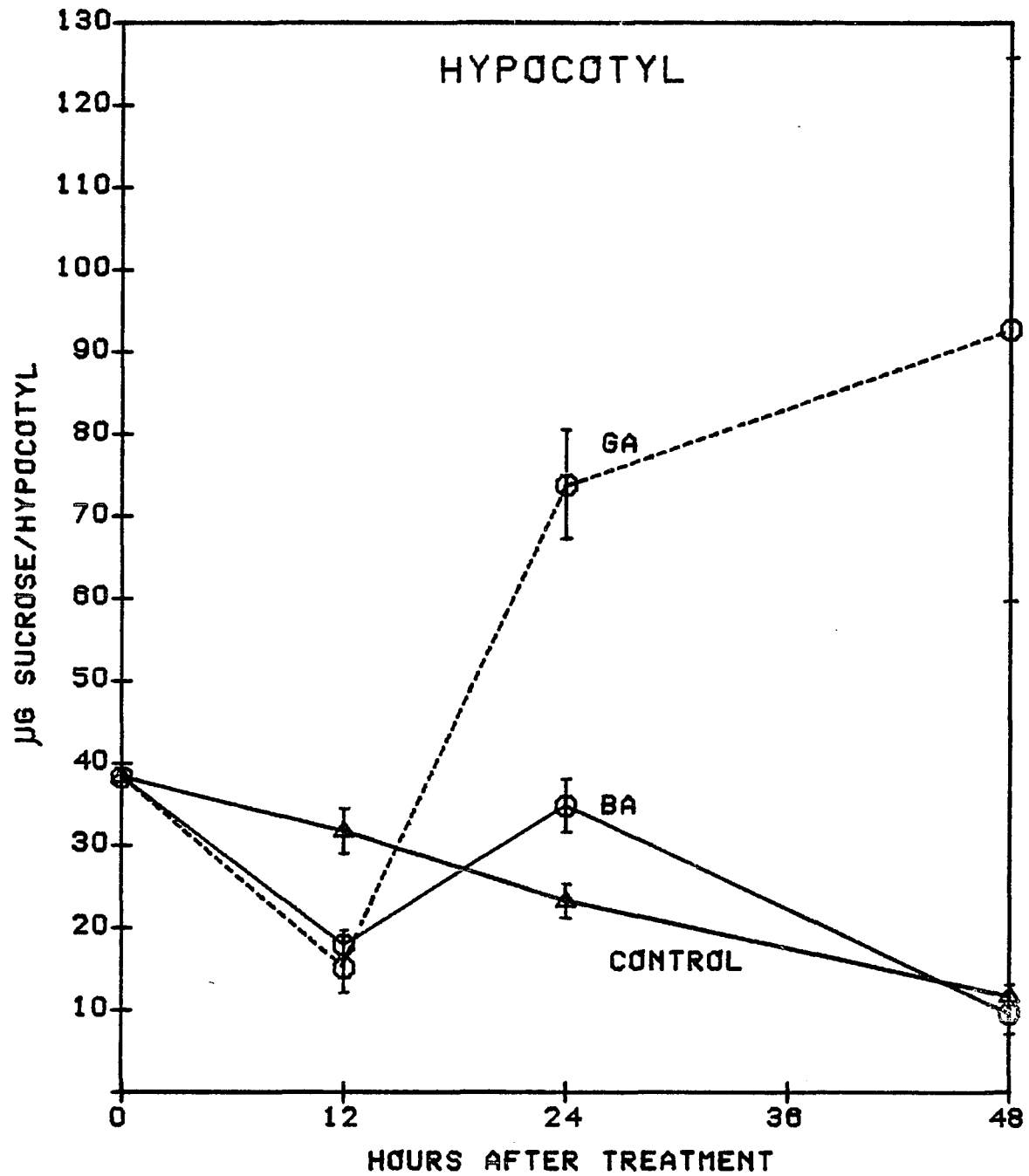


Fig. 16. Effect of BA and GA on sucrose (non-reducing sugars) content in intact hypocotyls. Each point represents the average of 4 replications. Vertical bars indicate standard errors.

amount of non-reducing sugars in GA-treated hypocotyls was nearly 9 times as high as that in hypocotyls of control or BA-treated seedlings.

Both BA and GA inhibited the maintenance of sucrose in intact roots (Fig. 17). By 48 h, sucrose was practically absent in roots of treated seedlings.

#### Cation Levels

BA and GA had no significant effect on the amount of  $K^+$  in the cotyledons, although the mean values obtained for BA-treated cotyledons were always higher than those of controls (Fig. 18). GA markedly promoted the  $K^+$  accumulation in the hypocotyls between 24 to 48 h, whereas BA was only slightly promotive at 12 and 48 h (Fig. 19). Both hormones inhibited  $K^+$  accumulation in the roots (Fig. 20).

In contrast with  $K^+$ ,  $Ca^{2+}$  and  $Mg^{2+}$  levels were very low. In most instances, the amounts of  $Ca^{2+}$  and  $Mg^{2+}$  in seedling organs were not significantly affected by BA or GA (Tables 2. and 3.). Small differences between treatments could be due to differential uptake from the absorbant wadding. Each absorbant wadding disc contained an average of 294 ug  $Ca^{2+}$ , 54 ug  $Mg^{2+}$ , and 50 ug  $K^+$ .

#### Relation of Solute Levels to Osmotic Potentials

A positive correlation existed between the observed concentration of osmotically active solutes and measured osmotic potentials in the cotyledons ( $R^2=0.95$ ). (Table 4.)

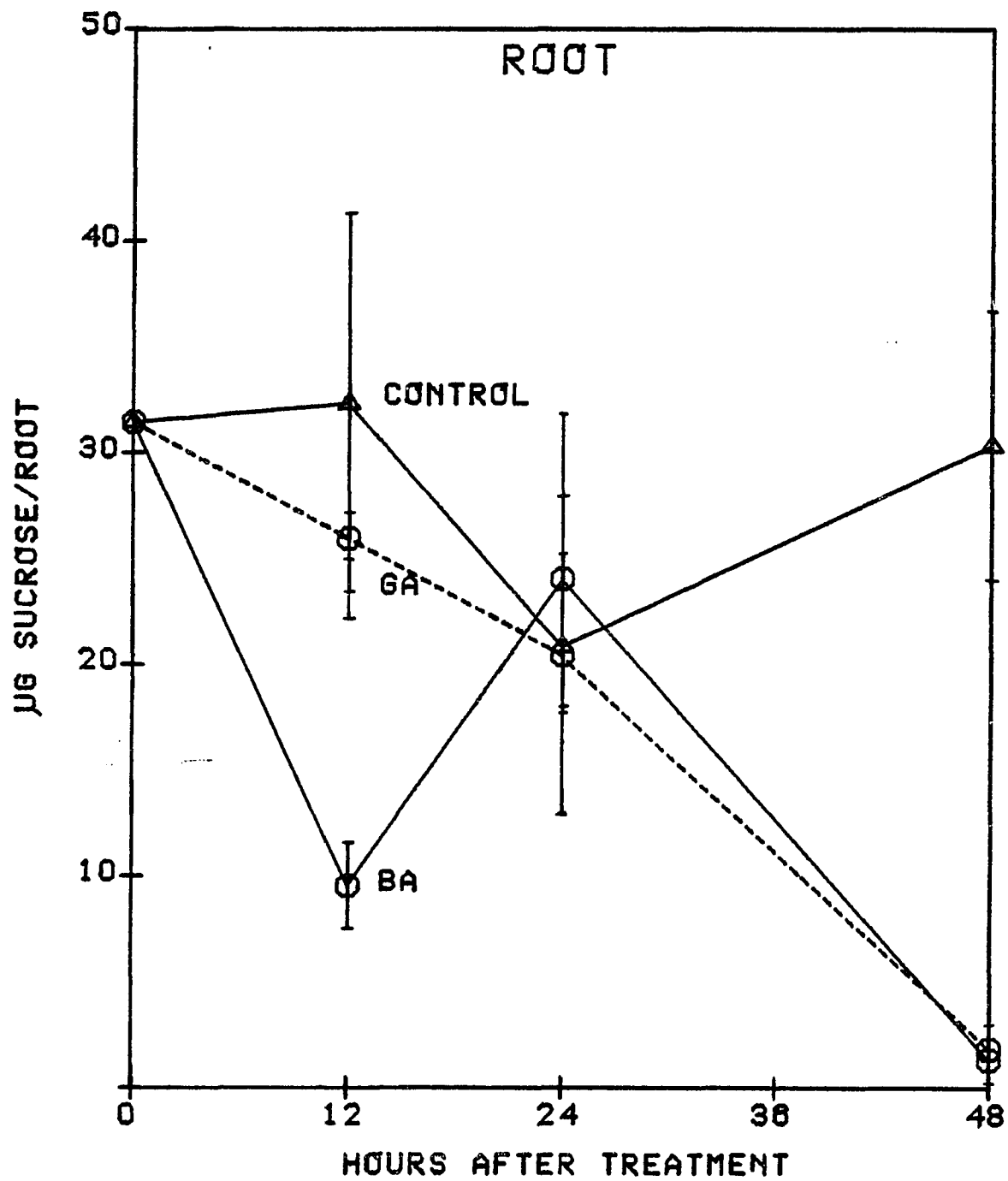


Fig. 17. Effect of BA and GA on sucrose (non-reducing sugars) content in intact roots. Each point represents the average of 4 replications. Vertical bars indicate standard errors.

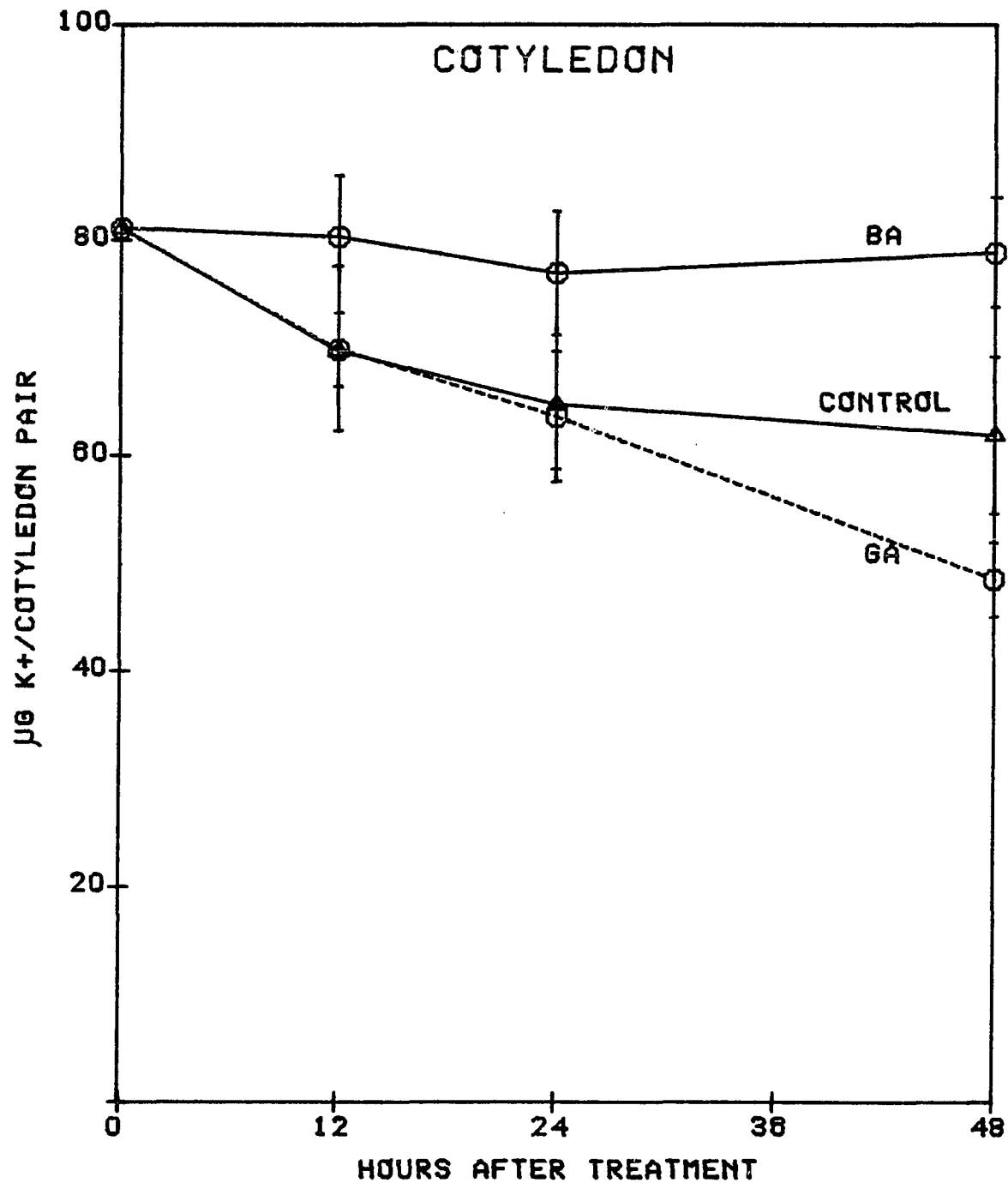


Fig. 18. Effect of BA and GA on K<sup>+</sup> content in intact watermelon cotyledons. Each point represents the average of 4 replications. Vertical bars indicate standard errors.

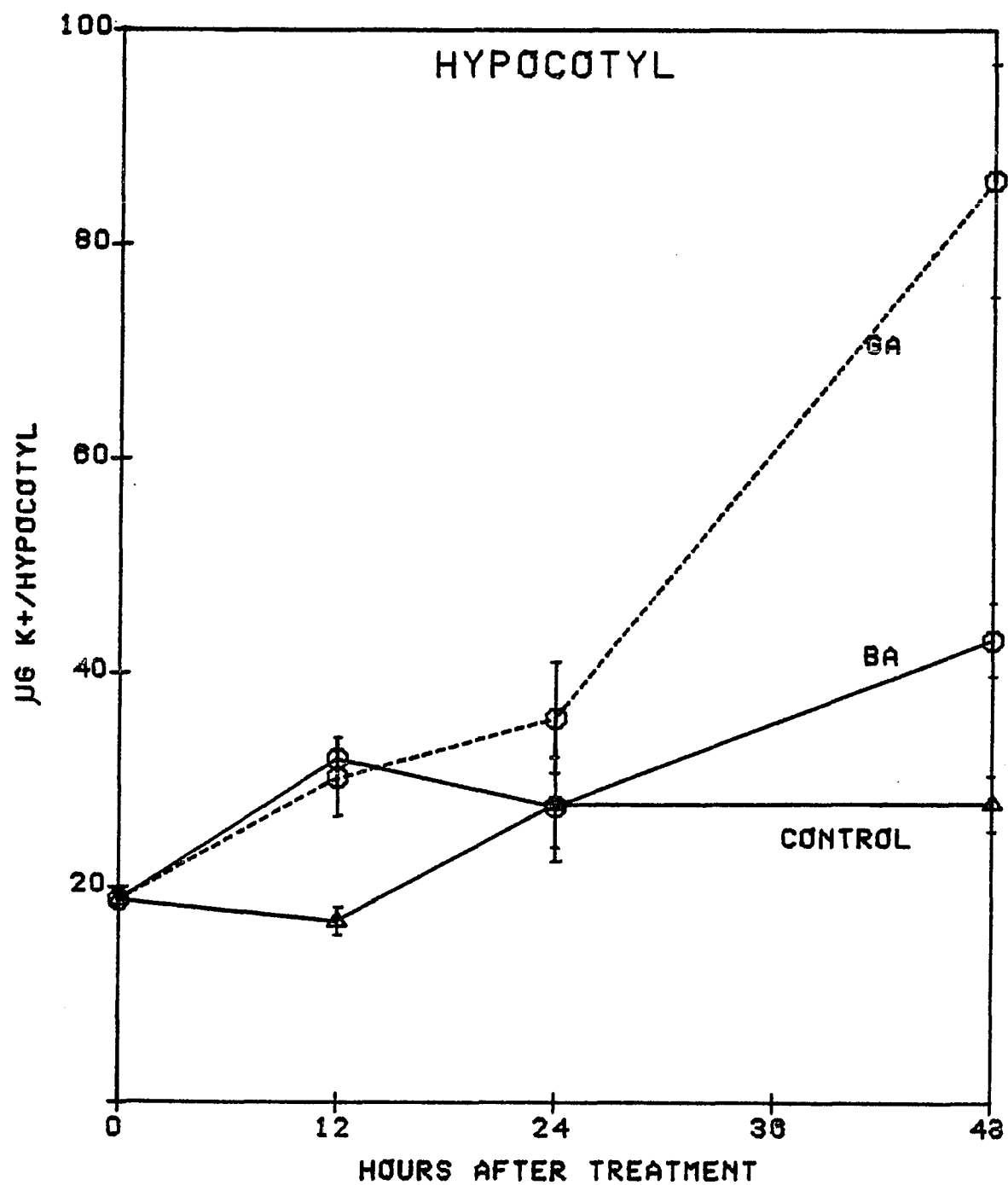


Fig. 19. Effect of BA and GA on  $\text{K}^+$  content in intact watermelon hypocotyls. Each point represents the average of 4 replications. Vertical bars represent standard errors.



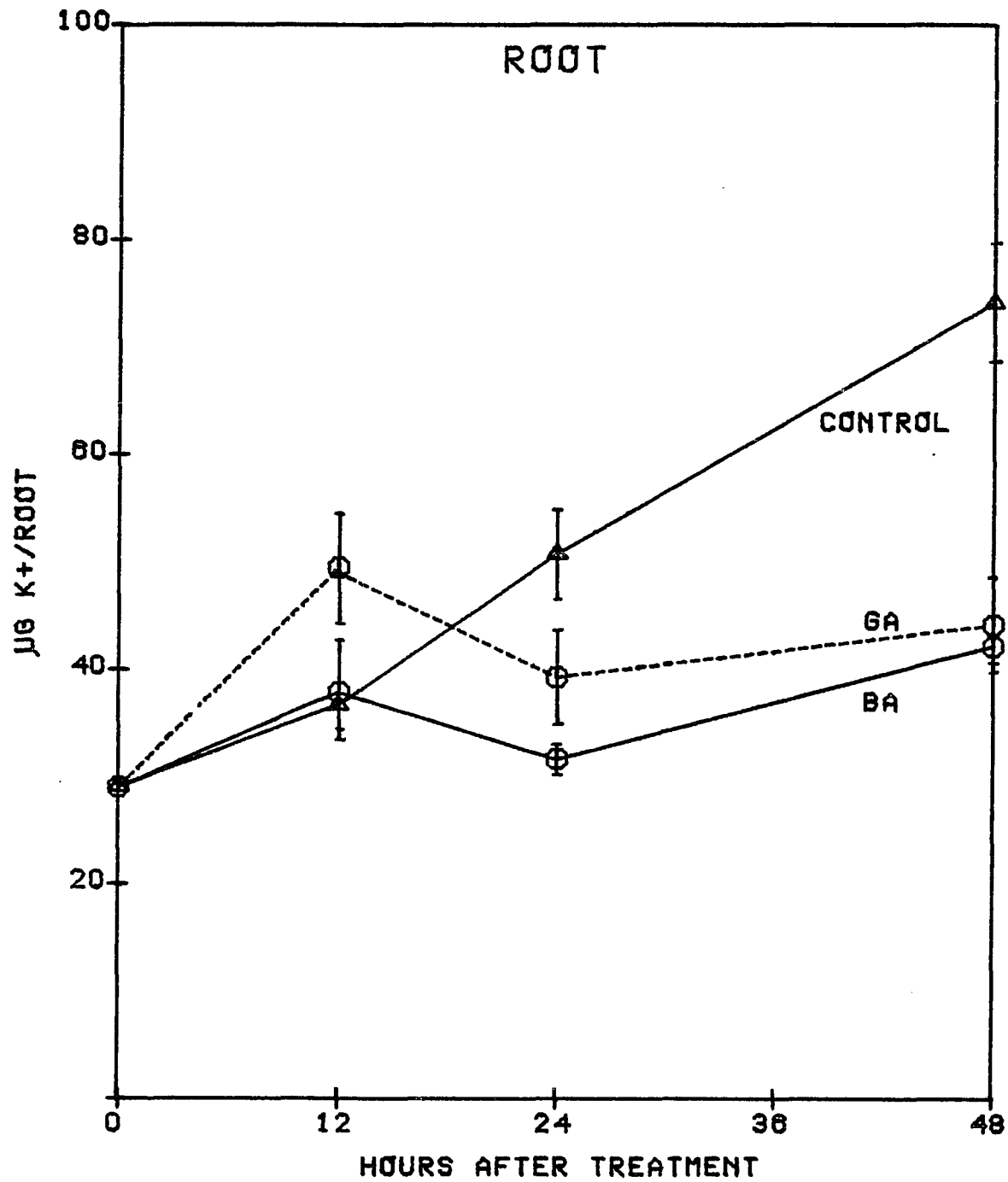


Fig. 20. Effect of BA and GA on K<sup>+</sup> content in intact watermelon roots. Each point represents the average of 4 replications. Vertical bars indicate standard errors.

Table 2. Effect of 0.2  $\mu\text{g}$  BA and 5  $\mu\text{g}$  GA on  $\text{Ca}^{2+}$  content in intact cotyledons, hypocotyls, and roots of dwarf watermelon seedlings.

Hours Following Treatment	Treatment	Cotyledon Pair	Hypocotyl	Root
$\mu\text{g}/\text{organ}^1$				
0	Control	5.6b	4.0b	4.0e
12	Control	6.8b	4.4b	4.8bcd
	GA	6.8b	5.2b	5.6ab
	BA	7.6ab	5.6b	4.4cde
24	Control	7.2b	4.8b	4.4cde
	GA	7.2b	5.2b	4.8bcd
	BA	6.4b	4.8b	4.0de
48	Control	9.2a	8.0a	6.0a
	GA	7.6ab	10.0a	5.2bc
	BA	6.8b	8.4a	4.4cde

<sup>1</sup>Mean separation (4 replicates) within columns according to Duncan's Multiple Range Test,  $P = 0.05$ .

Table 3. Effect of 0.2  $\mu\text{g}$  BA and 5  $\mu\text{g}$  GA on  $\text{Mg}^{2+}$  content in intact cotyledons, hypocotyls, and roots of dwarf watermelon seedlings.

Hours Following Treatment	Treatment	Cotyledon Pair	Hypocotyl	Root
			$\mu\text{g}/\text{organ}^1$	
0	Control	30.7c	2.4e	1.4b
12	Control	31.7c	3.1de	1.4b
	GA	39.1bc	4.1cd	1.9b
	BA	33.4c	3.4de	1.2b
24	Control	37.4bc	3.6cde	1.7b
	GA	39.8bc	5.0c	1.4b
	BA	33.8bc	4.3cd	1.4b
48	Control	46.8ab	7.0b	3.6a
	GA	52.6a	8.6a	1.4b
	BA	37.9bc	7.0b	1.2b

<sup>1</sup>Mean separation (4 replicates) within columns according to Duncan's Multiple Range Test,  $P = 0.05$ .

Table 4. Effect of 0.2  $\mu\text{g}$  BA and 5  $\mu\text{g}$  GA on total estimated osmotic components in intact cotyledons of dwarf watermelon seedlings.

Hours Following Treatment	Treatment	Osmotic Potential	Estimated <sup>1</sup> Osmotic Components
		(bars)	( $\mu\text{mol/g}$ fr wt)
0	Control	-11.7	154.3
12	Control	-10.4	131.7
	GA	-10.3	133.0
	BA	-6.5	95.0
24	Control	-9.0	126.9
	GA	-8.4	128.7
	BA	-6.9	87.2
48	Control	-7.8	92.6
	GA	-6.8	88.4
	BA	-5.2	64.9

<sup>1</sup>Values represent combined total for reducing and non-reducing sugars, potassium, calcium, and magnesium.

and in the hypocotyls ( $R^2=0.94$ ) (Table 5.). Thus, although BA increased the amount of solutes per cotyledon pair, there was a dilution of the cell sap during growth and a corresponding increase in osmotic potential.

GA increased the amount of soluble sugars and cations in the hypocotyl; whereas, BA had no measurable effect, but there was a dilution of cell sap in the hypocotyls of both GA- and BA-treated seedlings.

Table 5. Effect of 0.2  $\mu$ g BA and 5  $\mu$ g GA on total estimated osmotic components in intact hypocotyls of dwarf watermelon seedlings.

Hours Following Treatment	Treatment	Osmotic Potential	Estimated <sup>1</sup> Osmotic Components
		(bars)	( $\mu$ mol/g fr wt)
0	Control	-8.4	92.0
12	Control	-8.0	75.1
	GA	-7.8	84.3
	BA	-5.9	66.4
24	Control	-7.5	88.5
	GA	-5.9	60.7
	BA	-5.4	55.6
48	Control	-6.9	57.1
	GA	-4.2	38.1
	BA	-3.5	28.9

<sup>1</sup>Values represent combined total for reducing and non-reducing sugars, potassium, calcium, and magnesium.

## DISCUSSION

Both BA and GA promoted cotyledon expansion, increased hypocotyl elongation, and inhibited root growth in intact, light-grown dwarf watermelon seedlings; however, the magnitude and kinetics of BA- and GA- elicited changes in growth differed (Fig. 1,2,3,4).

The dosage of BA (0.2  $\mu\text{g}/\text{apex}$ ) in the above experiments was chosen for optimal hypocotyl elongation but, nevertheless, elicited a large increase in cotyledon expansion, an effect reported for several species (Banerji and Laloraya, 1967; Esashi and Leopold, 1969; Longo et al., 1978a; Narain and Laloraya, 1970; 1974). On the other hand, the high dosage of GA (5  $\mu\text{g}/\text{apex}$ ) needed for optimal hypocotyl elongation only slightly promoted cotyledon expansion, corroborating previous results of Rai and Laloraya (1967).

Cytokinins promote cotyledon expansion by affecting cell enlargement (Letham, 1971). Likewise, both BA and GA promote hypocotyl elongation in intact WB-2 seedlings by increasing cell size (Loy, 1980; Loy and Liu, 1974). Since cell enlargement requires the uptake of water by the vacuole (Heyn, 1940), a decrease in the osmotic potential and/or an increase in cell wall extensibility must accompany or precede water movement into the cell.

BA increased the amount of soluble sugars per cotyledon pair (Fig. 12,15), but there was a dilution of the osmotic constituents and a corresponding increase in the osmotic potential (Table 4.) during cotyledon expansion. Estimates of turgor pressure (Table 6.), calculated from differences between osmotic potentials and water potentials (Fig. 8), indicated that in expanding cotyledons of BA-treated seedlings a large decrease in turgor pressure occurred within 12 h following treatment, and by 24 h turgor pressure was approaching 0 bars. The calculated turgor pressures may be underestimated by as much as 11 to 16% since mixed cell sap was used for osmotic potential measurements and could be diluted by cell wall water (Wenkert, 1980). This could account for the continued expansion of BA-treated cotyledons at or near turgor pressures of 0 bars at 24 and 48 h. Although the measured osmotic potentials may be slightly magnified, there was a high correlation between the observed concentration of osmotically active solutes and measured osmotic potentials in cotyledons (Table 4.) and in hypocotyls (Table 5.).

The above results, coupled with the flaccid appearance of BA-treated cotyledons (personal observation) supports the conclusion of Longo et al. (1978a), that BA promotes cotyledon expansion in watermelon seedlings by increasing cell wall extensibility.



Table 6. Estimated turgor pressure values of intact cotyledons and hypocotyls treated apically with 0.2  $\mu$ g BA or 5  $\mu$ g GA.

Hours Following Treatment	Treatment	Cotyledons	Hypocotyl
		(bars) <sup>1</sup>	
0	Control	4.6 $\pm$ 0.4	5.0 $\pm$ 0.3
12	Control	3.5 $\pm$ 0.4	4.8 $\pm$ 0.1
	GA	2.2 $\pm$ 0.4	3.6 $\pm$ 0.4
	BA	1.4 $\pm$ 0.3	3.4 $\pm$ 0.4
24	Control	4.0 $\pm$ 0.4	4.2 $\pm$ 0.3
	GA	2.1 $\pm$ 0.2	1.9 $\pm$ 0.2
	BA	0.8 $\pm$ 0.4	3.2 $\pm$ 0.5
48	Control	3.1 $\pm$ 0.4	3.6 $\pm$ 0.3
	GA	2.5 $\pm$ 0.2	2.2 $\pm$ 0.2
	BA	0.3 $\pm$ 0.5	0.8 $\pm$ 0.5

<sup>1</sup>Estimated turgor pressures calculated from the difference between the water potential and osmotic potential. Each value represents the average of 10 replications for controls and 5 replications for GA and BA  $\pm$  standard errors.

Soluble sugar accumulation also increased in GA-treated cotyledons (Fig. 12,15), but in contrast with BA, GA had no significant effect on the osmotic potential of the cotyledons (Fig. 9), indicating that solute accumulation was keeping pace with water uptake. Since the water potential of GA-treated cotyledons decreased during the period of cotyledon expansion, extensibility changes can be inferred. Thus, GA-promotion of cotyledon expansion involved osmoregulation in addition to a lowering of the yielding threshold of the cell wall.

Whereas, GA often reverses genetic dwarfism (Brian and Hemming, 1955; Cooper, 1958; Denna, 1963; Phinney, 1956); exogenous application of cytokinins to intact plants usually inhibits stem elongation and promotes lateral expansion (Metvier and Paulilo, 1980; Sprent, 1968; Wittwer and Dedolph, 1963). The inhibitory effect of cytokinins may in part be attributed to their stimulation of ethylene production which can induce swelling of stems (Fuchs and Lieberman, 1968; Loy and Pollard, 1981). The kinetics of changes in hypocotyl fresh weight (Fig. 3) were closely parallel to the kinetics of hypocotyl elongation (Fig. 1) for control, BA-, and GA-treated seedlings, thus indicating only slight, if any, promotion of lateral swelling of hypocotyls at the levels of BA and GA used in this study.

Final cell size in mature hypocotyls is similar in GA- and BA-treated seedlings (compare Loy, 1980 with Loy and Liu, 1974), however, hypocotyl elongation is greater in

GA-treated seedlings because GA elicits a greater increase in cell number (Loy, 1980). Intact hypocotyls of WB-2 seedlings treated with optimum levels of GA respond slightly, or not at all, to exogenous applications of BA (Loy-unpublished results). These results suggest that GA and BA might affect cell elongation through similar mechanisms.

BA had no effect on the water potential of intact hypocotyls, but markedly increased the osmotic potential (Fig. 10). By 48 h, turgor pressure was approaching 0 bars (Table 6.); by this time 50% of BA-promoted growth is attained (Fig. 1). In contrast, the osmotic potential of GA-treated hypocotyls increased at a greater rate than that of control hypocotyls, but there was a corresponding increase in water potential between 24 to 48 h following treatment (Fig. 11). Therefore, although turgor pressure decreased (Table 6.), it apparently exceeded some yielding threshold of the cell to allow for continued hypocotyl growth exhibited by GA-treated seedlings.

These results indicate that both GA and BA stimulated hypocotyl elongation primarily by increasing cell wall extensibility. In contrast with BA, GA also promoted an increase in soluble sugars (Fig 13,16) and  $K^+$  (Fig. 19) in the hypocotyls which, in addition to maintaining cell turgor, might contribute to the synthesis of new cell wall material to maintain the properties of the cell wall for continued extension (Cleland, 1971). Thus, osmoregulation

plays an important role in GA-promotion of cell elongation in both cotyledons and hypocotyls. BA promoted both cotyledon expansion and hypocotyl elongation primarily by increasing cell wall extensibility, suggesting that solutes may be a limiting factor in BA-induced growth.

In comparison with control or GA-treated seedlings, BA decreased total translocation of metabolites out of the cotyledons (Fig. 5). The suppression of cotyledon dry weight loss is even more pronounced when respiration rates are considered. BA enhanced respiration of excised cotyledons throughout the 48 h measurement period, whereas GA had no effect (Table 1.). Both BA and GA increased the sink capacity of the hypocotyl (Fig. 6) at an expense to the roots (Fig. 7), however, maximum accumulation of dry matter into the hypocotyls was elicited by GA. The kinetics of changes in hypocotyl fresh and dry weights were similar in GA-treated seedlings; whereas, BA-promotion of hypocotyl fresh weight was measurable 30 to 36 h prior to an increase in dry weight.

After 48 h, the final dry weight of dark-grown seedlings did not differ significantly from that of light-grown seedlings (data not shown) indicating that the light intensity used in this study ( $4.2 \mu\text{E m}^{-2} \text{s}^{-1}$ ) was below the photosynthetic compensation point. Therefore, the observed increases in dry weight in the hypocotyls (Fig. 6) and roots (Fig. 7) must have arisen from the breakdown and subsequent translocation of stored cotyledonary reserves.

BA greatly accelerated lipid depletion in dark-grown excised watermelon cotyledons (Longo et al., 1978a) and cucumber cotyledons (Tsui et al., 1980). Accompanying this lipid breakdown was an increase in the amount of soluble sugars in both these organs. However in light-grown excised watermelon cotyledons, BA did not increase glyoxylate cycle enzymes and strongly accelerated their decay (Lampugnani et al., 1980). This is consistent with results of intact light-grown watermelon cotyledons, where total accumulation of soluble sugars was reduced in BA-treated seedlings (Fig. 21,22). On the other hand, cell enlargement requires the synthesis of new cell wall material (Cleland, 1971). Thus, in expanding cotyledons of BA-treated seedlings, solutes may be preferentially incorporated into the cell walls and therefore not available for translocation.

In contrast with BA, GA markedly increased total amounts of reducing (Fig. 21) and non-reducing sugars (Fig. 22). The increased accumulation of sugars was partitioned between the cotyledons (Fig. 12,15) and the hypocotyls (Fig. 13,16). Thus, it is likely that GA promoted lipid breakdown in addition to enhancing translocation. GA promotes lipid degradation in several species (Firn and Kende, 1974; Marriot and Northcote, 1977; Pinfield, 1968; Wrigley and Lord, 1977), and the activity of catalase, a marker enzyme for the glyoxylate cycle, is enhanced by GA during germination of light-incubated dwarf watermelon seeds (Evensen and Loy, 1978).

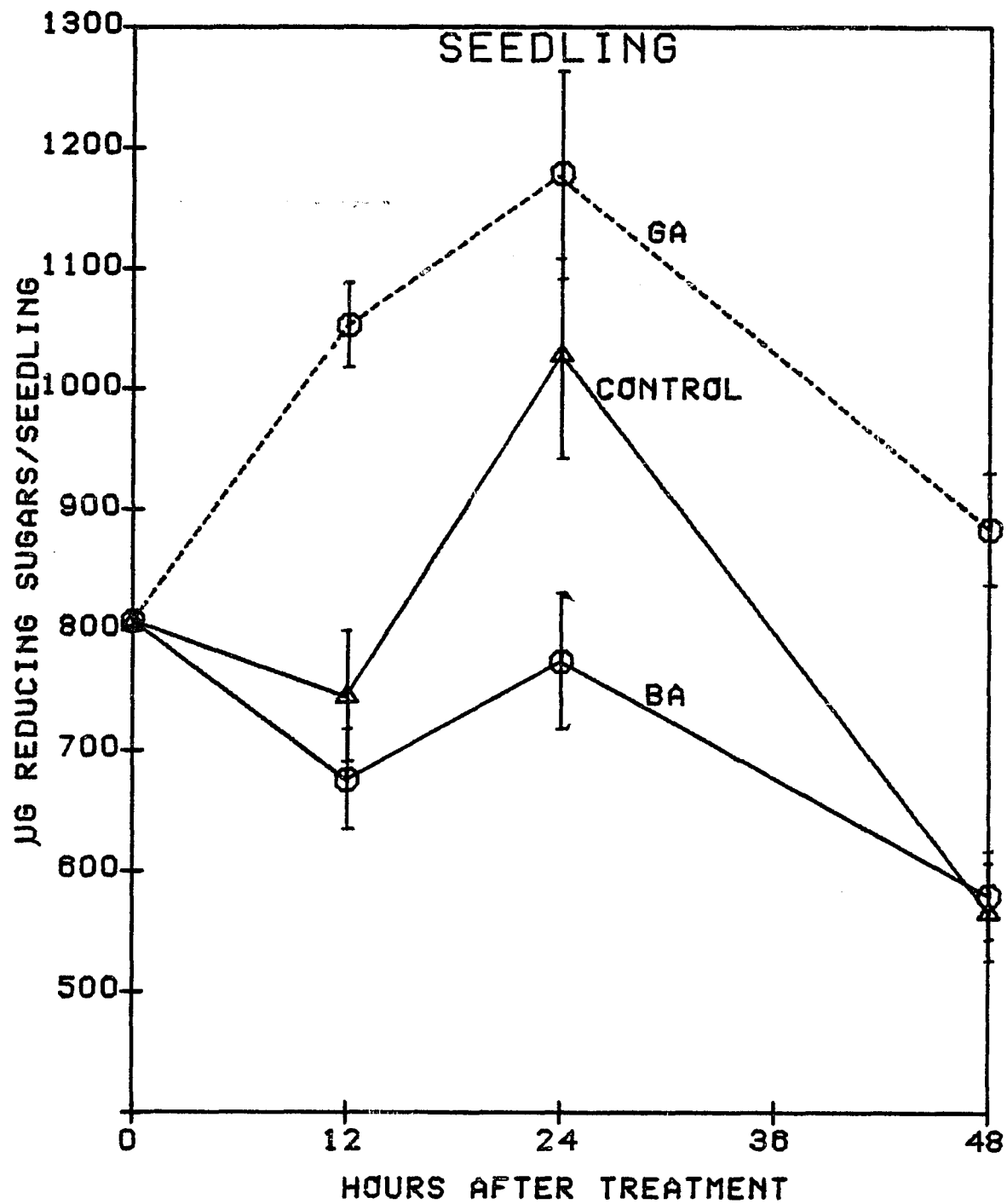


Fig. 21. Effect of BA and GA on total reducing sugar content in intact dwarf watermelon seedlings. Each point represents the average of 4 replications. Vertical bars indicate standard errors.

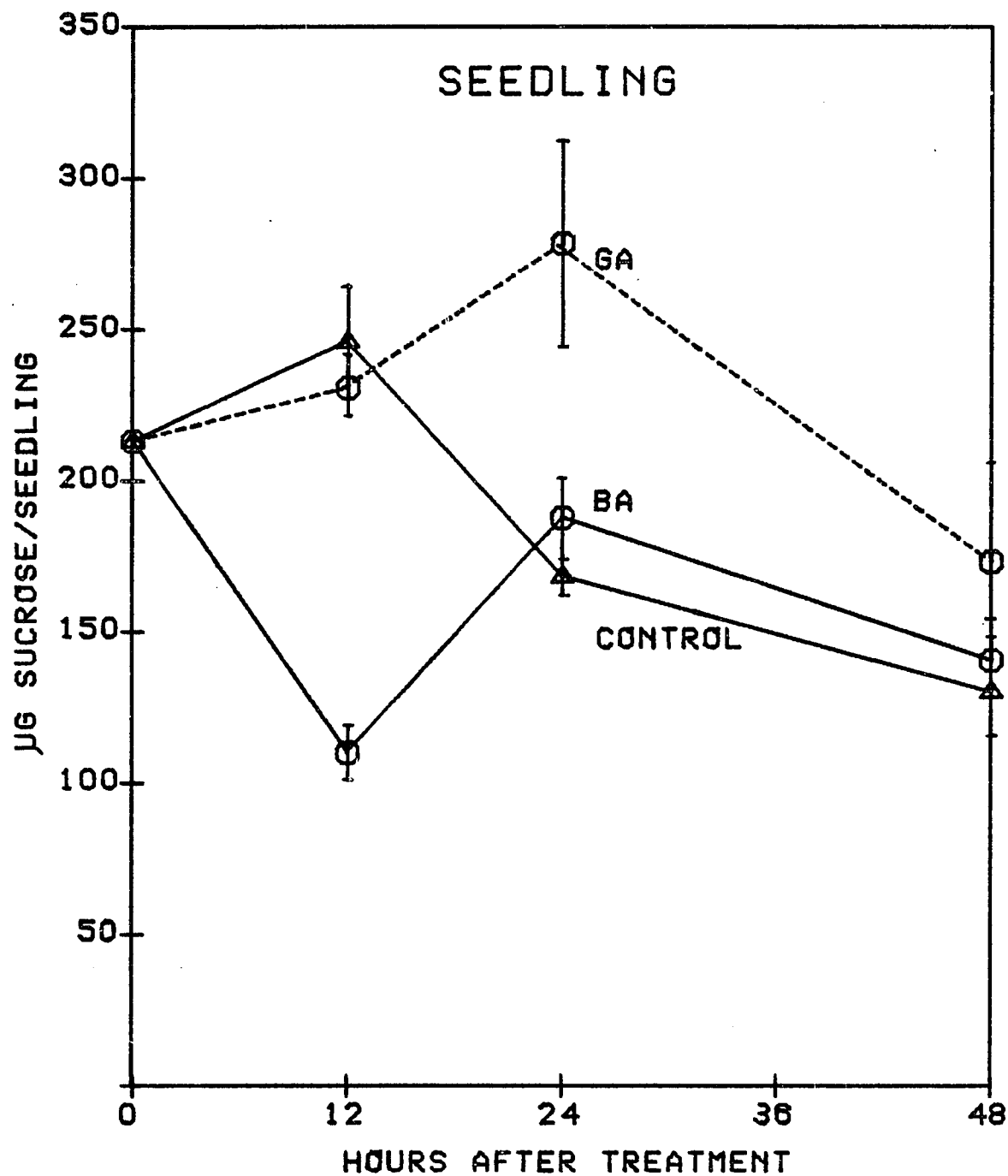


Fig. 22. Effect of BA and GA on total sucrose (non-reducing sugars) content in intact dwarf watermelon seedlings. Each point represents the average of 4 replications. Vertical bars indicate standard errors.

In many higher plants the main osmoticum is  $K^+$ , often associated with organic acids, whereas  $Ca^{2+}$  and  $Mg^{2+}$  are generally minor components (Cram, 1976). In intact dwarf watermelon seedlings,  $Ca^+$  and  $Mg^{2+}$  levels were very low in comparison with  $K^+$ , and in most instances were not significantly affected by BA or GA (Tables 2. and 3.). Neither BA or GA significantly affected  $K^+$  levels in the cotyledons (Fig. 18). GA markedly promoted  $K^+$  accumulation in the hypocotyls, whereas BA was only slightly promotive (Fig. 19). Neumann (1977) similarly reported that GA greatly increased  $K^+$  content in mesophyll cells of a dwarf maize mutant.



## CONCLUSIONS

The time course study of BA and GA effects on water and solute potential showed conclusively that GA-promotion of cotyledon expansion and hypocotyl elongation involves osmoregulation in addition to an increase in the extensibility of the cell wall; whereas, BA-stimulation of cotyledon expansion and hypocotyl growth rate is primarily due to a lowering of the yielding threshold of the cell wall.

A study on dry matter partitioning indicated that both BA and GA increase the sink capacity of the hypocotyl at an expense to the roots, however, in comparison with untreated or GA-treated seedlings, BA decreases total translocation of metabolites out of the cotyledons.

Analysis of soluble sugars and cations confirmed that GA promotes an increase in osmotically active solutes in the hypocotyl, which in addition to maintaining cell turgor, might contribute to the synthesis of new cell wall material to maintain the properties of the cell wall for continued extension.

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