INFLUENCE OF AMINO ACIDS ON GROWTH OF DATURA EMBRYOS IN CULTURE*

BY MARY E. SANDERS† AND PAUL R. BURKHOLDER

OSBORN BOTANICAL LABORATORY, YALE UNIVERSITY

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Investigations of the growth of *Datura* embryos in culture have shown that as early as the preheart stage, the embryos can be excised from the seeds and grown in an artificial medium containing only the major inorganic salts and sucrose. Such cultured embryos sometimes showed premature differentiation of root or plumule and grew to the seedling stage in less time than is required for the normal cycle of capsule ripening and seed germination. However, initial growth of the excised embryos, from preheart to mature embryo, never equalled that of the corresponding period of development in the seed which covers from 10 to 14 days (Sanders^{12, 13}). Addition of certain vitamins and organic acids did not markedly affect growth. However, addition of some organic complexes, notably coconut milk and malt extract, definitely increased the growth rate and favored normal development of the embryos (van Overbeek, Conklin and Blakeslee¹⁶; Blakeslee and Satina¹).

Studies on the nitrogen metabolism of higher plants have shown that many plants grown in sand culture, especially if deprived of inorganic nitrogen, can absorb and utilize certain sources of organic nitrogen. Legumes have been found better able to use organic nitrogen than other plants, such as wheat and barley (Virtanen and Linkola¹⁷). Plants furnished with external supplies of different amino acids have been found to have distinct morphological variations (Virtanen and Linkola¹⁷; Steinberg¹⁵). Tomato and tobacco seedlings deprived of inorganic sulphur were found able to use DL-methionine as a source of sulphur (Miller⁸). Though many amino acids can be utilized, toxicity is frequent and may be due either to specific acids or to unfavorable concentrations. Under controlled optimum conditions for growth, ammonium nitrogen has been shown to be utilized more rapidly than nitrate, but under usual conditions for growth, nitrates have proved to be the best external source of nitrogen for higher plants (Nightingale^{9, 10}).

With the development of tissue culture techniques, it has been possible to show that plant tissues, organs and embryos can assimilate inorganic nitrogen. On the whole, nitrates have again proved to be the best source of nitrogen although ammonium salts have been found available and, in some cases, a good source. For the most part, nitrogenous organic compounds have been toxic to plant tissue cultures, but there are exceptions. With tomato roots, White¹⁹ found that glycine with thiamin satisfactorily

replaced yeast extract in the nutrient. For growth of bacteria-free crown-gall tissue of sunflower, Riker and Gutsche¹¹ found urea to be the only organic nitrogenous compound approaching the effectiveness of nitrate. Some of the amino acids (DL-alanine, L-arginine HCl, L and DL-aspartic acid and L-glutamic acid) did prove to be available for growth and were not toxic when present with nitrate. In the culture of orchid embryos, addition of amino acids to a medium containing nitrate failed to improve growth and frequently showed toxicity (Withner²⁰; Spoerl¹⁴). Spoerl found that arginine and aspartic acid under certain conditions were as effective as ammonium nitrate, but used in addition to ammonium nitrate, they showed no stimulation of growth. He found that the effect of the amino acids depended upon concentration, light conditions, species of orchid and age of embryos. In studying the problem of rooting in asparagus stem tips in artificial culture, Galston⁴ found that glutamine and arginine stimulated growth but were ineffective for root formation. Kent and Brink⁶ observed that casein hydrolysate,† an amino acid complex, prolonged embryonic growth and inhibited germination of *Hordeum* embryos *in vitro*. Although the majority of amino acids supplied to plant cultures have proved to be growth inhibitors, alanine, arginine, aspartic acid, glutamic acid and glycine have proved beneficial to growth under certain conditions.

Materials and Methods.—Plants used in these investigations were *Datura innoxia* (S.I. 115) and *D. stramonium* (S.I. 1) (from the collection of Dr. A. F. Blakeslee) and were grown under greenhouse conditions. Self embryos were selected at the pre- or early heart stage (longitudinal axis, 0.1 to 0.25 mm.), dissected out of the seeds and grown in artificial liquid nutrients. Ten embryos per nutrient were used. Experiments ran for twelve days and results for individual media are expressed as growth values, final average length/original average length.

Basal media consisted of water, major inorganic salts, trace elements, vitamins and sucrose. The water used was double-distilled in Pyrex glass. The major salt formula was composed to obtain the following proportions of ions, 8 K : 1 Ca : 1 Mg and 4 NO₃ : 3 PO₄ : 3 SO₄. The following salts were used with "1" representing 1 millimolecular weight per liter of solution: 2 KNO₃, 1 Ca(NO₃)₂, 2 KH₂PO₄, 1 NaH₂PO₄, 1 MgSO₄ and 2 K₂SO₄. In some of the experiments, the inorganic nitrogen was increased so that the ratios were 8 K : 1 Ca : 1 Mg and 6 NO₃ : 3 PO₄ : 1 SO₄, and the formula became 4 KNO₃, 1 Ca(NO₃)₂, 3 KH₂PO₄, 1 MgSO₄ and 1 KCl. Increased nitrate, however, did not affect the growth values to any extent. The former formula gave growth values of 6.21 for *D. innoxia* and 5.5 for *D. stramonium*; the latter, 6.45 for *D. innoxia* and 4.88 for *D. stramonium*. Trace elements provided were in p. p. m.: B, 0.1; Mn, 0.1; Zn, 0.3; Cu, 0.1; Mo, 0.1; Fe, 0.5. Vitamin concentrations were in p. p. m.:

FIGURE 1

Response of preheart embryos of two species of *Datura* to casein hydrolysate with cysteine and tryptophane added to the basal medium.

thiamin HCl, 0.1; pyridoxine HCl, 0.8; niacin, 0.8. Sucrose was used at 2 per cent for *D. innoxia* and 4 per cent for *D. stramonium* (Sanders¹³). All nutrients were adjusted to between pH 5.7 and 6.0.

Response of Embryos to Casein Hydrolysate with Cysteine and Tryptophane Added to the Basal Medium.—Casein hydrolysate together with cysteine and tryptophane (each at 6.67 mg. per 100 mg. casein) was added to the

basal medium at 0, 25, 50, 100, 200, 400, 800 and 1600 p. p. m. Growth values for this series are represented in the graphs of figure 1. Growth of embryos of both species increased markedly especially in the range 100 to 800 p. p. m. Embryos of *D. stramonium* were much more stimulated than those of *D. inoxia*. At 400 p. p. m., the concentration which gave best growth in both species, growth values were 44.8 and 16.1, respectively.

FIGURE 2

Photographs ($\times 5\frac{1}{2}$) of *Datura* embryos grown for twelve days in a nutrient series to which casein hydrolysate with cysteine and tryptophane was added. A-H, embryos of *D. inoxia*. I-P, embryos of *D. stramonium*. A and I, in basal medium; B and J, in basal medium plus casein hydrolysate at 25 p. p. m.; C and K, at 50 p. p. m.; D and L, at 100 p. p. m.; E and M, at 200 p. p. m.; F and N, at 400 p. p. m.; G and O, at 800 p. p. m.; H and P, at 1600 p. p. m.

At 25 and 50 p. p. m., growth values were not much larger than for the basal medium, and at 800 and 1600 p. p. m., they decreased markedly in *D. stramonium* and somewhat in *D. inoxia*. Representative embryos from these series are shown in figure 2. The amino acid complex increased embryonic differentiation as well as the actual size of the embryos. After twelve days in basal medium, embryos were approximately 1 mm. in length. Embryos grown to this size in the seed have arrived at the late heart or

early torpedo stage and have well-defined cotyledons and no root primordia (Sanders¹²). In basal medium, *D. innoxia* embryos showed retarded cotyledonary development and frequently premature root primordia, and *D. stramonium* embryos showed little differentiation although cotyledon primordia were sometimes discernible (figure 2, A and I). With addition of casein hydrolysate, even at the lower concentrations, embryonic differentiation became more normal. Throughout the *D. innoxia* series, cotyledons were shorter in relation to the hypocotyl than are cotyledons of embryos grown in the seed. Abnormalities and root primordia appeared when casein hydrolysate was present, but less frequently and on much larger embryos than in basal medium.

Response of Embryos to Mixtures of Amino Acids and to Ten Single Amino Acids Added to Basal Medium.—In an attempt to identify the growth factors in casein hydrolysate, embryos were grown in a series of nutrients

TABLE 1

COMPOSITION OF AMINO ACID MIXTURES ADDED TO BASAL MEDIUM USED FOR GROWTH OF *Datura* EMBRYOS. (ACIDS MARKED WITH AN ASTERISK WERE THE COMPONENTS OF "MODIFIED MIXTURES" A AND B)

MIXTURE A		MIXTURE B	
Amino Acid	Concentration, p. p. m.	Amino Acid	Concentration p. p. m.
DL-alanine	6.0	*L-cystine	1.5
*L-arginine	12.0	*L-histidine	9.0
*L-aspartic acid	18.0	L-hydroxyproline	1.5
L-cysteine	20.0	DL-isoleucine	15.0
L-glutamic acid	60.0	L-leucine	15.0
Glycine	1.5	*DL-methionine	9.0
*L-lysine	18.0	*DL-serine	18.0
DL-phenylalanine	12.0	DL-threonine	12.0
*L-proline	24.0	L-tryptophane	20.0
L-tyrosine	18.0	*DL-valine	24.0

containing mixtures of amino acids or individual amino acids. Three controls were run for the series, basal medium, basal medium with NH_4Cl at 108 p. p. m. (an amount roughly equivalent to the amino nitrogen in casein hydrolysate at 300 p. p. m.), and basal medium plus casein hydrolysate at 300 p. p. m. with L-cysteine and L-tryptophane. Concentrations of individual amino acids (with the exception of cysteine and tryptophane which were used at the previous concentrations given above), were based on the amino acid analysis of casein hydrolysate (Harrow⁵). Amounts approximately equivalent to the content of the acids in hydrolyzed casein at 300 p. p. m. were determined. The amino acids were divided into two groups of ten each; mixture A included those acids which have been found beneficial for plant growth under certain conditions (Burkholder, Castle and Smith³; Riker and Gutsche¹¹; Spoerl¹⁴; White¹⁹); mixture B, the remaining ten acids which have shown no effect or have been

inhibitory to growth. The composition of mixtures A and B are given in table 1. Acids marked with an asterisk are those which were included in modified mixtures A and B. Amino acids of mixture A were also added singly to the basal medium at the same concentrations as in the mixture. Data from this series of nutrients are represented by the graphs in figure 3. In both species, addition of ammonium chloride increased growth somewhat suggesting an ammonium salt might be beneficial in the basal medium, but it did not approach the stimulatory effect of casein hydrolysate. Addition of mixtures A and B together in the same medium closely reproduced the results obtained with casein hydrolysate, strongly suggesting that the growth stimulus is due to the amino acids present in hydrolysed

FIGURE 3

Response of preheart embryos of two species of *Datura* to ammonium chloride and various organic nitrogen sources added to the basal medium.

casein. Addition of B alone gave no increase in growth. Addition of A alone gave a growth value for *D. innoxia* embryos equal to that for A and B, suggesting that the entire stimulatory effect of casein hydrolysate might be contributed by the amino acids in A. The growth value for *D. stramonium* embryos on basal medium with A alone was less than half that with A and B suggesting that an interaction of the acids in the two mixtures is necessary to produce the growth stimulus obtained with casein hydrolysate. Addition of single amino acids also gave different results for the two species. With *D. innoxia* embryos, cysteine alone gave a definite increase in growth; aspartic acid showed a slight increase; alanine, phenylalanine, tyrosine

and perhaps glycine showed inhibition. With *D. stramonium* embryos, arginine, aspartic acid, lysine and proline increased growth; alanine and perhaps tyrosine showed inhibition. Modified mixture A was made up of the four acids stimulatory to *D. stramonium* embryos when added separately. Comparison of the growth value for modified A with that for A indicates that at least some of the six omitted acids were functional in promoting growth either individually or by interaction with other acids. Mixture B added with modified A decreased rather than increased embryo growth. Omission of six acids from A has apparently upset the amino acid balance which favored growth. Modified mixture B was made up by omitting five of the acids in B which have shown inhibition in other investigations. Modified B added to the basal medium with modified A almost doubled the growth value for modified A alone. This group of nine acids provided a more favorable balance. By careful selection and combination it might be possible to reproduce the growth stimulus of the original 20 acids by a balanced mixture of fewer acids. Embryos of *D. innoxia* were grown on basal medium plus aspartic acid and cysteine, the two acids which gave increased growth for this species, but the growth value was less than for either of the acids alone. Balance of amino acids is apparently an important factor determining the influence of these compounds upon growth of *Datura* embryos.

Amino acids which individually stimulated growth, four for *D. stramonium* and two for *D. innoxia*, were also tested at three concentrations each; arginine at 5, 10 and 20 p. p. m.; aspartic acid, cysteine, lysine and proline at 10, 20 and 40 p. p. m. Growth values for *D. stramonium* embryos increased from 3.3 to 6.8 with changes in arginine concentration from 5 to 20 p. p. m., decreased from 10.0 to 6.5 with changes in lysine concentration from 10 to 40 p. p. m., and were fairly constant with the changes in the concentrations of aspartic acid and proline (4.4 to 4.8 with the former and 6.1 to 5.6 for the latter). Growth values for *D. innoxia* embryos were 6.9, 8.9 and 6.9 with aspartic acid at 10, 20 and 40 p. p. m., and increased from 5.0 to 13.7 with cysteine from 10 to 40 p. p. m. Interaction of the acids casts some doubt on the possibility of determining optimum concentrations for growth by addition of varying amounts of single acids. This procedure does provide some evidence concerning the effect of different concentrations of the individual amino acids on growth and differentiation.

It has been mentioned that addition of casein hydrolysate to the basal medium favored normal differentiation of the embryos in culture. Mixtures A and B together were equally beneficial to normal differentiation. The other mixtures and the single amino acids were less effective for normal differentiation, but certain characteristics appeared. Although growth values for *D. innoxia* embryos in basal medium with A plus B and with A alone were very similar, the patterns of growth differed. With A plus B,

cotyledons of the embryos were short in proportion to the hypocotyls as with casein hydrolysate (figure 2, *B-H*). With A alone, hypocotyls were short in proportion to cotyledons. Addition of mixture A resulted in the same type of growth in *D. stramonium* embryos whereas with A plus B, cotyledon-hypocotyl proportions were normal as in figure 2, *L-O*. Modified A had the same effect as A, but modified A plus modified B produced embryos with proportionally short cotyledons. Differentiation as well as growth is influenced by the interaction of the amino acids present. Some single amino acids had recognizable effects on type of growth. Alanine interfered with cotyledon and hypocotyl differentiation in *D. innoxia* embryos but allowed formation of premature root primordia and even slight growth in the region of the plumule. Cysteine at 20 and 40 p. p. m. increased the number of root primordia and actual root growth in *D. innoxia* embryos. Lysine, especially at 10 p. p. m., had a similar effect on *D. stramonium* embryos. It also contributed to the formation of translucent, watery tissue instead of the firm, white tissue of healthy embryos beyond the heart stage. Changes in the balance of amino acids can apparently enhance premature differentiation of embryos to seedlings as well as prevent it.

Discussion.—Most plants naturally assimilate inorganic nitrogen, and amino acids are generally poor sources of nitrogen for plants in sand cultures and for many plant cultures *in vitro*. The metabolism of plants and of plant parts which synthesize their own organic nitrogenous compounds is apparently upset by supplying an excess of these substances. To study the effects of amino acids on growth in the presence of inorganic nitrogen, other than inhibition or abnormalities, it is necessary to culture plant tissues, organs or embryos which are able to use to advantage an external source of amino acids in addition to their own capacity for synthesis. Sunflower tissue (Riker and Gutsche¹¹) and orchid embryos (Withner²⁰; Spoerl¹⁴) with an adequate supply of inorganic nitrogen are apparently able to synthesize their necessary amino acids as rapidly as they can use them. Asparagus stem tips (Galston⁴), tomato root tips (White¹⁹), immature *Hordeum* embryos (Kent and Brink⁶) and immature *Datura* embryos can apparently use amino acids faster than some of these compounds can be synthesized in such isolated structures. It may well be that *in vivo* amino acids are supplied to such structures by other parts of the plant. Without an external source of amino acids, young *Datura* embryos grown in culture require a much longer time to mature than in the seed or with a mixture of amino acids in the nutrient.

The behavior of mixtures indicates that the influence of these acids on growth results from interaction rather than accumulation of the individual effects of single acids. Evidence of such interaction has been found with numerous microorganisms. For example, Bonner² demonstrated in a

single gene mutant of *Neurospora* that a block in the conversion of keto isoleucine to isoleucine resulted in an accumulation of the former which inhibited the conversion of keto valine to valine, thus causing a deficiency of two amino acids. Inhibition of growth in *Lactobacillus arabinosus* by glycine can be counteracted by the addition of D-alanine (Kobayashi, Fling and Fox⁷). Inhibition of growth in *Streptococcus bovis* by isoleucine, leucine, threonine or norleucine can be counteracted by valine, glutamic acid, methionine or cystine. Inhibition caused by equal amounts of phenylalanine and tyrosine, which singly had no effect, can be counteracted by tryptophane, glutamic acid or cystine. It is suggested that inhibiting amino acids may block the synthesis of another acid and that the counteracting acid may be the acid blocked or an acid from which it can be formed (Washburn and Niven¹⁸).

Single additions of amino acids have been found to have characteristic effects on the morphology of plants. This phenomenon suggests that the balance of amino acids probably changes during development and that such changes may influence growth and differentiation. Steinberg¹⁵ suggests changes in the balance of amino acids as the basis for the production of morphological symptoms of certain plant diseases. Some of the problems of differentiation, relative growth and dormancy, would appear to be closely related to amino acid metabolism.

The effect on embryo growth of casein hydrolysate and of the mixture of amino acids approximating the composition of casein hydrolysate is similar to the beneficial effects of other organic complexes, namely coconut milk and malt, which have been found to stimulate growth of *Datura* embryos, favor embryonic differentiation and retard premature germination (van Overbeek, Conklin and Blakeslee¹⁶; Blakeslee and Satina¹). The varied activities of such natural complexes may well be due to their component amino acids.

Differences in the effects of amino acid mixtures and of single acids on embryos of two separate species suggest that the amino acid content of female parent tissues and of endosperm may be important in interspecific crosses, especially crosses in which embryos are first formed but later abort. An attempt to correlate similarity of amino acid requirements of self embryos of the parent species and of the hybrid embryos with species crossability might prove more successful than earlier attempts to correlate species crossability with similarity of sucrose and inorganic salt requirements of the embryos (Sanders¹³).

Summary.—Early heart embryos of *Datura innoxia* and *D. stramonium* (0.1 to 0.25 mm. in length) were dissected out of the seeds and grown in artificial culture in basal medium alone and also with additions of casein hydrolysate, mixtures of amino acids, and single amino acids.

Casein hydrolysate with cysteine and tryptophane markedly improved growth and embryonic differentiation in the range 100 to 800 p. p. m.

A mixture of 20 amino acids approximating their occurrence in casein hydrolysate at 300 p. p. m. resulted in growth equal to that with casein hydrolysate indicating that the amino acids are responsible for the growth stimulus provided by hydrolyzed casein.

Differences in growth values for lesser mixtures and for individual amino acids, which were for the most part well below those for the mixture of 20 acids, provide evidence that the growth stimulus of the combination of 20 acids results from physiological interactions rather than from the summation of effects of individual acids.

Characteristic patterns of differentiation appeared with some mixtures and with some individual acids.

The species differed in their responses both to mixtures and to single amino acids.

Balance of amino acids appears to be a controlling influence on both growth and differentiation of plant embryos.

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† Present address, Department of Botany, University of Wisconsin, Madison, Wisconsin.

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ON THE STABILITY AND INSTABILITY OF SHOCK WAVES

BY T. Y. THOMAS

INDIANA UNIVERSITY AND GUGGENHEIM AERONAUTICAL LABORATORY

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The following communication contains a criterion for the stability and instability of plane stationary shock waves (compression shocks) attached to the vertex V of a pointed obstacle O in a uniform field of flow.¹ Behind the shock line the flow may be rotational and non-isentropic; viscosity and thermal conductivity are assumed zero in this theory. It will be assumed that the shock line and boundary of the obstacle are analytic

FIGURE 1

curves and that the functions u_α (velocity components), p (pressure) and ρ (density) defining the flow are analytic in the region behind, and also along the shock line.