

GLUTAMINE AND ASPARAGINE AS NITROGEN SOURCES FOR THE GROWTH OF PLANT EMBRYOS *IN VITRO*: A COMPARATIVE STUDY OF 12 SPECIES

By A. H. G. C. RIJVEN*

[Manuscript received March 28, 1956]

Summary

Subsequent to earlier work on *Capsella bursa-pastoris* (L.) Moench. embryos of nine plant families, monocotyledons and dicotyledons, excised from young developing ovules were grown in a "sitting drop" culture.

For each species, the embryonic growth in length was compared in a control medium, i.e. without nitrogen, and in media containing L-glutamine and L-asparagine. In all cases the growth in glutamine was superior.

Additional data on *Capsella* are presented which deal with the growth-concentration relationships of glutamine and asparagine. Glutamine increasingly stimulates growth up to 600 mg/l. Asparagine stimulates growth at 10 mg/l but becomes inhibitory at higher concentrations.

The inhibition of growth with asparagine (400 mg/l) was also found in *Arabidopsis thaliana* (L.) Heynh. and *Reseda odorata* L. However, at this concentration, asparagine proved stimulating in *Sisymbrium orientale* L., *Cleome viscosa* L., *Medicago tribuloides* Desr., and *M. orbicularis* All., in *Anagallis arvensis* L., *Datura stramonium* L., *Chenopodium album* L., and *Allium cepa* L.

An earlier suggestion about the nature of asparagine-induced inhibition is partly revised.

I. INTRODUCTION

Evidence that glutamine plays a more important role than asparagine in the anabolic activities in higher plants has accumulated during the last decade (Steward and Thompson 1954). The present paper adds to this evidence and advocates the use of glutamine in a special type of plant tissue culture. It may be reiterated that the nitrogen nutrition of young embryos is exceptional, as they are apparently unable to utilize nitrate nitrogen. The evidence for this stems from *in vitro* growth studies of embryos excised from developing ovules in which the effect of different nitrogen sources was investigated.

Rijven (1952) has demonstrated that torpedo-shaped embryos of *Capsella bursa-pastoris* grown on a basal medium with nitrate as sole nitrogen source show a rapidly decreasing growth rate and that this was not due to exhaustion of a component of the medium. Nevertheless, under these conditions the nitrogen source was apparently limiting growth, for addition of amino acids greatly reduced this decline of the growth rate.

More direct information is available from an investigation on *Medicago orbicularis*. A comparison made between growth of embryos cultured on a basal medium containing either calcium nitrate or calcium chloride showed no difference in growth. However, these embryos again respond markedly to the addition of a number of amino acids to the medium (Rijven, unpublished data).

* Division of Plant Industry, C.S.I.R.O., Canberra, A.C.T.

Embryos of *C. bursa-pastoris* and *M. orbicularis* respond to ammonium compounds but much less than to a number of amino acids. For both species, glutamine added as sole nitrogen source to the medium has been found superior to any other amino acid tried, and also to glutamic acid added in nitrogen-equivalent amounts. Paris *et al.* (1953) have reported similar responses by *Datura stramonium*. They also noted that the presence or absence of nitrate in the basal medium does not affect growth.

In a previous study (Rijven 1955) on the *in vitro* growth of embryos of *Capsella bursa-pastoris*, a marked contrast was observed in growth of embryos supplied either with glutamine or asparagine as nitrogen source, viz. a stimulation by glutamine and a retardation by asparagine, both as compared with a control to which no organic nitrogen was added. Also, asparagine reduced the growth when added to media containing glutamine. In those experiments only one concentration of asparagine was used, viz. 400 mg/l. Additional data concerning growth-concentration relationships for glutamine and asparagine are presented here.

Though this work has revealed that asparagine stimulates growth slightly at 10 mg/l, the opposing effects of glutamine and asparagine at higher concentrations have been confirmed. This surprising result invoked interest in whether or not similar growth responses would be found with these amides in other plants.

The choice of species was partly determined by the desire to cover some of the old scheme "glutamine- and asparagine-families" of Schulze as listed by Chibnall (1939). At the same time the 12 species tried cover nine plant families belonging to seven orders, two of them monocotyledons. Special attention has been given to the Rhoeadales.

II. METHODS AND MATERIALS

As previously described (Rijven 1952) our method is a rather short period test (1-4 days) of growth in length of embryos excised from ovules and explanted in a modified "sitting drop" culture technique. The culture cells contain up to 20 embryos which are suspended in separate drops (about 30 μ l) of liquid medium between two glass slides, to enable the measurement of their lengths by means of a microscope fitted with an eyepiece micrometer. Handling of the drops and of the embryos is done with sterilized braking-pipettes which were first introduced into the field of enzymic ultramicroanalysis by Linderstrøm Lang and Holter (1941). It may be stressed that these implements proved very useful. To allow heat sterilization, the capillaries were fitted with a high melting resin ("Araldite", from CIBA, Basle) instead of paraffin.

Previous results have shown that the growth of young plant embryos is very much affected by the quantity and quality of the nitrogen source. In *C. bursa-pastoris* a concentration of 400 mg/l of glutamine gives growth that is close to the maximal response. Lower concentrations limit the growth. This concentration of L-glutamine has therefore been adopted for experiments on embryos of other species. The same (c. equimolar) concentration is used in the L-asparagine series (asparagine is weighed as L-asparagine monohydrate; both amides were products of L. Light & Co. Ltd., England). In some cases other concentrations are used as well. For each species also

a series with basal medium only, i.e. without a nitrogen compound added, was used as a control. However, except for the experiments on *Capsella* a minute amount of ammonium molybdate was present in the basal medium.

The basal medium consisted of 1 l. glass-distilled water to which was added 120 g sucrose, and as salt nutrients K_2HPO_4 , 87 mg, KH_2PO_4 , 340 mg, $CaCl_2$, 55 mg, $MgSO_4 \cdot 7H_2O$, 49 mg, $MnSO_4$, 0.45 mg, H_3BO_3 , 0.062 mg, $ZnSO_4 \cdot 7H_2O$, 0.287 mg, $CuSO_4 \cdot 5H_2O$, 0.024 mg, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.12 mg, ferric citrate, 15 mg, were added. The media, with pH 6.0 ± 0.2 , were sterilized by filtration through sintered-glass filters.

The cultures were incubated at $26 \pm 0.2^\circ C$ in a dark incubator and measurements made at 24-hr intervals.

The following species were tested in the order shown:

Medicago tribuloides Desr., December 1953, from Dickson Experimental Station, A.C.T.

Medicago orbicularis All., May 1954, from glass-house cultures

Anagallis arvensis L., red variety, March 1954, from roadside

Datura stramonium L., April 1954, from glass-house cultures

Chenopodium album L., May 1954, from roadside

Hordeum vulgare L., smooth awns, January 1955, from glass-house cultures

Allium cepa L., January 1955, from garden

Arabidopsis thaliana (L.) Heynh., March 1955, from glass-house cultures

Reseda odorata L., April 1955, from garden

Cleome viscosa L., October 1955, from glass-house cultures

Sisymbrium orientale L., November 1955, from roadside

Capsella bursa-pastoris (L.) Moench., November, December 1955, from roadside.

In general, up to 20 torpedo-shaped embryos between 0.5–1 mm in length were taken as initial material for each treatment.

The collection and dissection of the necessary number of ovules sometimes takes several hours so that embryos are not all treated in exactly the same way. This necessitates randomizing in the control medium in which the embryos are also dissected, prior to distribution over the treatments.

In such experiments it is unavoidable that the starting material, the excised embryos, vary in initial length. In general, the data for any one species was found to fit the formula:

$$y = a + bx, \quad \dots\dots\dots(1)$$

where y = length increment, and x = initial length. The regression of length increment on initial length is not the same in different species or often in different treatments.

In statistical analysis and in presentation of the data a pooled regression slope (regression coefficient b) has been used for each experiment except when the separate slopes of the different treatments were significantly different. Differences between y values, estimated at the mean of all x values, and found significant at the 5 per cent. level at least, are recorded in the final columns of the tables.

The treatments were not replicated in sets of culture cells, but in most cases the main issue of the experiments has been checked by repeating them, e.g. where unexpected results were obtained.

III. RESULTS

The data on the Rhoadales are presented prior to those of other plant groups; the latter follow in the chronological sequence in which the experiments were done as indicated in Section II.

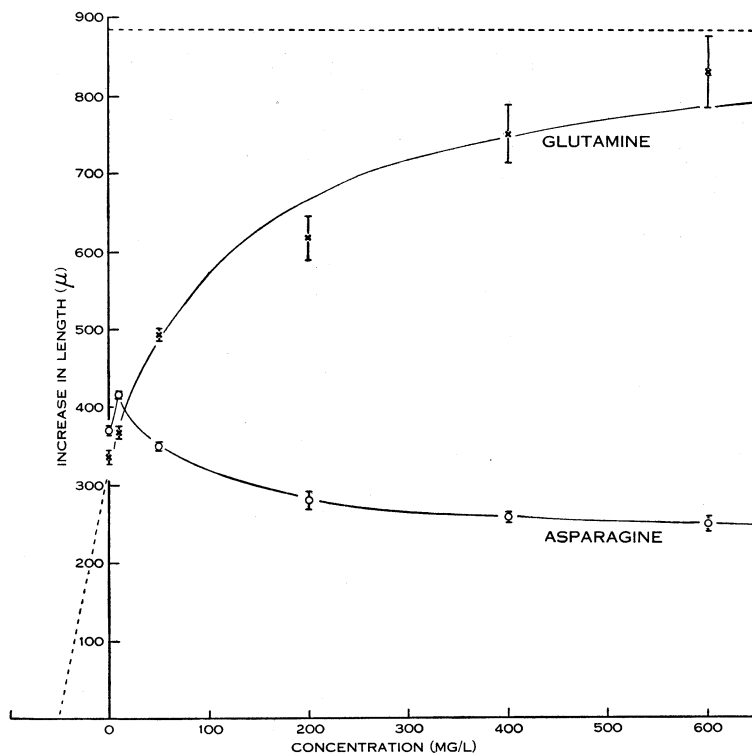


Fig. 1.—*Capsella bursa-pastoris*. Relation between growth value of embryos of 623.3μ after 48 hr and various concentrations of glutamine and asparagine. The line drawn for glutamine, based on Table 1, represents the theoretical function $y = (884.1c' + 44116.59)/(c' + 131.14)$, which is described more fully in Section IV. The line drawn for asparagine, based on Table 2, is empirical. The vertical lines at the dots represent the standard errors.

(a) *Capsella bursa-pastoris*, *Arabidopsis thaliana*, and *Sisymbrium orientale*

(i) *Capsella bursa-pastoris*.—In earlier work (Rijven 1955) the concentration of glutamine and asparagine used was frequently 400 mg/l. The choice of this concentration was based mainly on previous experience with different amino acids for which the effect of concentration had been tested (Rijven 1952). However, since the discovery of the contrasting effects of glutamine and asparagine, information on the effect of different concentrations of these particular amino acids has been lacking. A summary of some experiments to fill this gap is presented in Figure 1 and Tables 1 and 2.

Glutamine, as was expected, is progressively growth-promoting up to the highest concentration tried. In the mathematical treatment in Section IV the data

TABLE 1
EFFECT OF GLUTAMINE CONCENTRATION ON GROWTH OF EMBRYOS OF CAPSELLA BURSA-PASTORIS

Corrected estimates of length increment (y) of embryos at the mean initial length (x) of 623.3μ cultured for 48 hr in basal medium (control) and in five media to which glutamine was added. The regression coefficient denotes the regression of length increment on initial length. The statistical treatment of the data in this and all succeeding tables is explained in the text

Treatment No.	Glutamine Concn. (mg/l)	No. of Observations	Regression Coefficient	y in μ at $x = 623.3 \mu$	Treatments Differing Significantly from the Particular Treatment
1	Control	17	0.324	337.2	$1 < 2, 3, 4, 5, 6$
2	10	15	0.282	369.0	$1 < 2 < 3, 4, 5, 6$
3	50	15	0.339	491.3	$1, 2 < 3 < 4, 5, 6$
4	200	13	0.446	615.5	$1, 2, 3 < 4 < 5, 6$
5	400	17	1.027	751.9	$1, 2, 3, 4 < 5$
6	600	13	2.739	830.7	$1, 2, 3, 4 < 6$

of Table 1 have been shown to fit the Michaelis-Menten formula. In Figure 1 the curve drawn for glutamine represents the results of those calculations.

TABLE 2
EFFECT OF ASPARAGINE CONCENTRATION ON GROWTH OF EMBRYOS OF CAPSELLA BURSA-PASTORIS

Corrected estimates of length increment (y) of embryos at the initial length (x) of 623.3μ cultured for 48 hr in basal medium (control) and in five media to which asparagine was added. The mean initial length was 632.6μ . The regression coefficient denotes the regression of length increment on initial length

Treatment No.	Asparagine Concn. (mg/l)	No. of Observations	Regression Coefficient	y in μ at $x = 623.3 \mu$	Treatments Differing Significantly from the Particular Treatment
1	Control	9	0.266	369.9	$2 > 1 > 4, 5, 6$
2	10	15	0.266	414.3	$2 > 1, 3, 4, 5, 6$
3	50	14	0.266	350.3	$2 > 3 > 4, 5, 6$
4	200	14	0.266	281.9	$1, 2, 3 > 4$
5	400	18	0.266	259.7	$1, 2, 3 > 5$
6	600	9	0.266	251.4	$1, 2, 3 > 6$

Asparagine is stimulating at 10 mg/l, but, as concentration increases, becomes inhibitory, growth rate falling off to a steady level. The stimulation at 10 mg/l was not expected. The consequence of this finding, which was confirmed in two more

experiments, is that an earlier attempt to explain asparagine-induced inhibition has to be revised (see Section IV). Nevertheless, the contrasting behaviour of

TABLE 3
EFFECTS OF ASPARAGINE AND GLUTAMINE ON GROWTH OF EMBRYOS OF ARABIDOPSIS THALIANA
Corrected estimates of length increment (y) of embryos at the mean initial length (x) of $285.8\ \mu$ cultured for 48 hr in basal medium (control) and in media containing asparagine or glutamine.
The regression coefficient denotes the regression of length increment on initial length

Treat- ment No.	Treatment	No. of Observa- tions	Regression Coefficient	y in μ at $x = 285.8\ \mu$	Treatments Differing Significantly from the Particular Treatment
1	Control	15	0.472	190.1	$2 < 1 < 3$
2	Asparagine, 400 mg/l	10	0.472	128.5	$2 < 1, 3$
3	Glutamine, 400 mg/l	7	0.472	366.1	$2, 1 < 3$

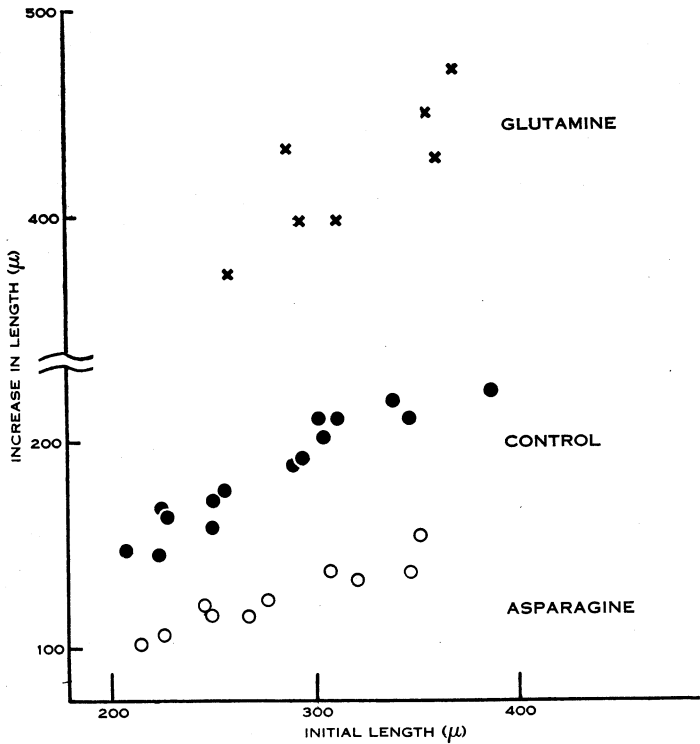


Fig. 2.—*Arabidopsis thaliana*. Dot diagram of observations on individual growth values after 48 hr and initial lengths of embryos grown in control media and in glutamine (400 mg/l) and asparagine (400 mg/l).

Capsella embryos at higher concentrations of the homologous amides remains a peculiar phenomenon.

(ii) *Arabidopsis thaliana*.—This species was chosen in order to discover whether the results obtained on *C. bursa-pastoris* would be valid for other Cruciferae.

A. thaliana produces 30–40 ovules per ovary with embryos of about equal size. Dissection is easy. Torpedo-shaped embryos cover a range from 200–450 μ . Actually this small size is an advantage in our culture drop technique. The fact that flowering plants are easily obtained all year round together with the other properties makes this species a promising test object for more thorough studies.

The results obtained in a number of experiments support the expectations. Glutamine is strongly stimulating, but the interesting point is the inhibition found in the asparagine series when compared with the control series (Table 3 and Fig. 2). This result is in good agreement with the experience on *C. bursa-pastoris*.

(iii) *Sisymbrium orientale*.—Not all Cruciferae follow the same response pattern as *Capsella* and *Arabidopsis*, for in the case of *S. orientale*, a definite stimulation by asparagine was found, increasing with increasing concentration up to an optimal value of 200 mg/l (Table 4).

TABLE 4

EFFECT OF ASPARAGINE CONCENTRATION ON GROWTH OF EMBRYOS OF *SISYMBRIUM ORIENTALE*
Corrected estimates of length increment (y) of embryos at the mean initial length (x) of 552.7 μ cultured for 48 hr in basal medium and in four media containing different asparagine concentrations. The regression coefficient denotes the regression of length increment on initial length

Treatment No.	Asparagine Concn. (mg/l)	No. of Observations	Regression Coefficient	y in μ at $x = 552.7 \mu$	Treatments Differing Significantly from the Particular Treatment
1	Control	19	0.608	321.3	1 < 2, 3, 4, 5
2	10	13	0.746	361.9	1 < 2 < 4, 5
3	50	14	0.548	387.6	1 < 3 < 4, 5
4	200	16	1.070	496.5	1, 2, 3, 5 < 4
5	400	20	0.958	452.0	1, 2, 3 < 5 < 4

This result was not due to an irregularity in the manipulation or to an impurity in the asparagine used, for in another experiment in which *Sisymbrium* together with *Capsella* embryos were tested with the same medium, the findings for both were completely consistent with earlier results.

(b) *Reseda odorata*

This species belongs to the Resedaceae and as this family is claimed to be closely related to the Cruciferae, it was thought that asparagine-induced inhibition of embryo growth was likely to occur here also.

As a technical difficulty it should be mentioned that the integument of the ovules in the required young stages is already considerably lignified so that for the excision of embryos extra care is needed to prevent damage.

Glutamine again proved strongly stimulating. In five out of six experiments a significant inhibition by asparagine was found (Table 5). The inhibition was

most evident during the first 3 days of culture. In some experiments longer incubation led to a recovery from the inhibition and in two experiments there was evidence for stimulation after 6 days. In one of these no initial inhibition was found. Attempts to reproduce this "adaptive behaviour" failed.

TABLE 5

EFFECT OF ASPARAGINE ON GROWTH OF EMBRYOS OF RESEDA ODORATA

Corrected estimates of length increment (y) of embryos at the mean initial length (x) of 486.4μ cultured for 48 hr in basal medium (control) and in medium containing asparagine. The regression coefficient denotes the regression of length increment on initial length

Treatment No.	Asparagine Concn. (mg/l)	No. of Observations	Regression Coefficient	y in μ at $x = 486.4 \mu$	Treatments Differing Significantly from the Particular Treatment
1	Control	31	0.368	288.6	$2 < 1$
2	400	16	0.368	212.9	$2 < 1$

(c) *Cleome viscosa*

This species of the Capparidaceae was tested for the same reason as *R. odorata*, viz. to check if the asparagine-induced inhibition could be found in more families of the Rhoeadales.

TABLE 6

EFFECTS OF ASPARAGINE AND GLUTAMINE ON GROWTH OF EMBRYOS OF CLEOME VISCOSA

Corrected estimates of length increment (y) of embryos at the mean initial length (x) of 614.1μ cultured for 48 hr in basal medium (control) and in three media containing asparagine or glutamine. The regression coefficient denotes the regression of length increment on initial length

Treatment No.	Treatment	No. of Observations	Regression Coefficient	y in μ at $x = 614.1 \mu$	Treatments Differing Significantly from the Particular Treatment
1	Control	16	0.032	293.4	$1 < 2, 3, 4$
2	Asparagine, 400 mg/l	20	0.793	378.4	$1 < 2 < 3, 4$
3	Asparagine, 600 mg/l	12	0.420	451.5	$1, 2 < 3 < 4$
4	Glutamine, 400 mg/l	16	0.149	980.5	$1, 2, 3 < 4$

The ovules and embryos of *C. viscosa* are very much like those of *R. odorata*; in dissection, therefore, the same precautions against damage are necessary.

Glutamine again proved strongly stimulating. Asparagine also was repeatedly found significantly stimulating though much less so (Table 6). This stimulation in *Cleome* is in contrast with the inhibition in *Reseda* notwithstanding the fact that the ovules appeared so much alike.

(d) *Medicago tribuloides* and *M. orbicularis*

These species, belonging to the Leguminosae, were chosen partly as representing the classical "asparagine type" of plant. The characteristic helical pods do not contain

TABLE 7

EFFECTS OF ASPARAGINE AND GLUTAMINE ON GROWTH OF EMBRYOS OF *MEDICAGO TRIBULOIDES*
Corrected estimates of length increment (y) of embryos at the mean initial length (x) of 637.3μ cultured for 48 hr in basal medium (control) and in media containing asparagine or glutamine, The regression coefficient denotes the regression of length increment on initial length

Treat- ment No.	Treatment	No. of Observa- tions	Regression Coefficient	y in μ at $x = 637.3 \mu$	Treatments Differing Significantly from the Particular Treatment
1	Control	14	0.355	291.8	$1 < 2, 3$
2	Asparagine, 400 mg/l	19	0.480	401.9	$1 < 2 < 3$
3	Glutamine, 400 mg/l	17	0.609	518.4	$1, 2 < 3$

many ovules. Isolation proved difficult with *M. tribuloides*, but was easier with *M. orbicularis*, so this species was used more in the later stages. As Table 7 shows,

TABLE 8

EFFECTS OF ASPARAGINE AND GLUTAMINE ON GROWTH OF EMBRYOS OF *MEDICAGO ORBICULARIS*
Corrected estimates of length increment (y) of embryos at the mean initial length (x) of 631.0μ cultured for 48 hr in basal medium (control) and in five media containing asparagine or glutamine or both. The regression coefficient denotes the regression of length increment on initial length

Treat- ment No.	Treatment	No. of Observa- tions	Regression Coefficient	y in μ at $x = 631.0 \mu$	Treatments Differing Significantly from the Particular Treatment*
1	Control	5	0.108	240.9	$1 < 2, 3, 4, 5, 6$
2	Glutamine, 400 mg/l	15	0.795	535.9	$1, 3 < 2 < 6$
3	Asparagine, 400 mg/l	15	0.411	358.8	$1 < 3 < 2, 4, 5, 6$
4	Asparagine, 2000 mg/l	18	0.683	540.3	$1, 3 < 4 < 6$
5	Glutamine, 400 mg/l, + asparagine, 400 mg/l	16	0.775	571.8	$1, 3 < 5 < 6$
6	Glutamine, 400 mg/l, + asparagine, 2000 mg/l	16	0.850	630.1	$1, 2, 3, 4, 5 < 6$

* Treatments 2, 4, and 5 do not differ significantly.

the growth responses of *M. tribuloides* to the three treatments are well differentiated after 48 hr incubation, and these results lead to the conclusion that in this species glutamine induces more growth than asparagine, and the latter more than the control.

Essentially the same results were repeatedly found for *M. orbicularis*. When a series containing glutamine and asparagine together (total nitrogen concentration thereby doubled) was included in the experiment beside the other series, the response of this series was not significantly different from that given by glutamine alone (see Table 8). Asparagine does not interfere with glutamine utilization as it actually does at this concentration in some Cruciferae. The effect of asparagine alone can be increased by increasing its concentration up to 2000 mg/l. The result of this treatment (No. 4 of Table 8) again is not significantly different from glutamine at a concentration of 400 mg/l. When, however, the amides were added together in these concentrations (treatment No. 6 of Table 8) the growth response was higher than that resulting from each alone.

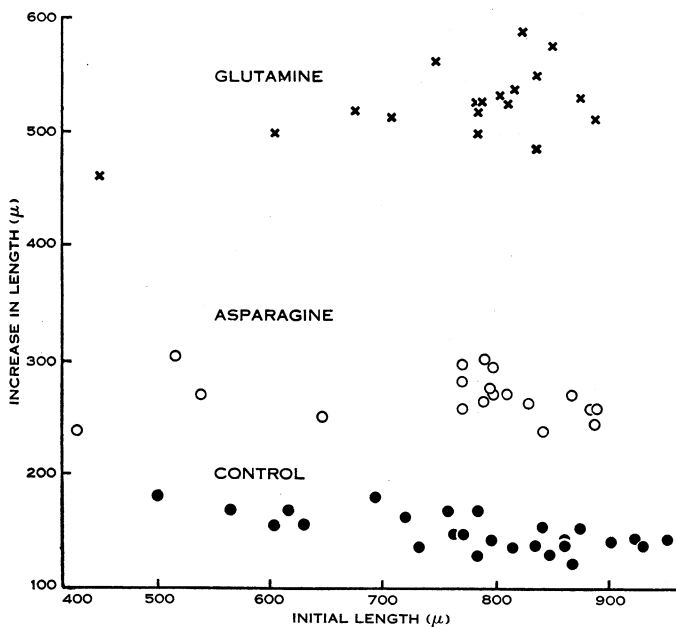


Fig. 3.—*Anagallis arvensis*. Dot diagram of observations on individual growth values after 48 hr and initial lengths of embryos grown in control media and in glutamine (400 mg/l) and in asparagine (400 mg/l).

(e) *Anagallis arvensis*

In *A. arvensis* (Primulaceae) the embryos remain straight and torpedo-shaped even in the mature seed. The excision of embryos from the ovules proved difficult, since the embryos were surrounded by a thick milky endosperm, which adhered to their surface.

The absence of correlation between growth and initial length which we have observed only in this species is noteworthy (see Fig. 3). This absence of significant correlation could mean that in these torpedo-shaped embryos growth is already restricted to certain well-defined meristematic zones which produce new, non-dividing cells at a constant rate. Therefore, in this species, it was sufficient to calculate the mean length increments and the standard errors (see Table 9). The same situation as

in the medics is found here, viz. glutamine affording more growth than asparagine and the latter being stimulative also. Addition of both amides together resulted in a growth response not significantly different from that given by glutamine alone.

TABLE 9

EFFECTS OF ASPARAGINE AND GLUTAMINE ON GROWTH OF EMBRYOS OF *ANAGALLIS ARVENSIS*
Mean length increment of embryos grown for 48 hr in basal medium (control) and in media containing asparagine or glutamine or both

Treat- ment No.	Treatment	No. of Observa- tions	Mean Initial Length (μ)	Mean Length Increment (μ)	Treatments Differing Significantly from the Particular Treatment
1	Control	18	601.6	167.6 (S.E. 5.4)	1 < 2, 3, 4
2	Asparagine, 400 mg/l	18	551.7	293.6 (S.E. 6.1)	1 < 2 < 3, 4
3	Glutamine, 400 mg/l	12	602.9	478.8 (S.E. 10.6)	1, 2 < 3
4	Glutamine, 400 mg/l, + asparagine, 400 mg/l	18	560.6	484.2 (S.E. 10.1)	1, 2 < 4

(f) *Datura stramonium*

This species, belonging to the Solanaceae, has been the object of extensive studies which are reviewed by Rappaport (1954). Testing of the relevant amides on this species has already been made by Paris *et al.* (1953). However, their culture method differs considerably from ours and their observations concern a longer period of incubation (8 days). These authors state that L-glutamine has a favourable effect on young embryos, while the effect of L-asparagine is only limited.

The results of one of our experiments are summarized in Table 10. Again glutamine stimulates most, but asparagine is also certainly stimulating on the growth of these small embryos, which is in good agreement with the results of the forementioned authors. In another experiment, asparagine and glutamine were added together; there was no discernible difference of growth stimulation as compared with glutamine alone.

Rietsema, Satina, and Blakeslee (1953) determined 8 per cent. sucrose to be optimal for small embryos. In contrast to the basal medium applied in the other species, 10 per cent. sucrose was used here, and in the second experiment described 8 per cent. was used. In both cases the growth rate was very high with four- to five-fold increases of length in 2 days for embryos initially 250 μ in length.

(g) *Chenopodium album*

This species belongs to the Chenopodiaceae which is listed by Chibnall (1939) as one of Schulze's "glutamine" families.

Collecting of embryos of the right stage is rather laborious as the ovaries contain only one ovule each. The young torpedoes are only slightly curved, but in measuring they are assumed to be straight, i.e. the length of the straight line connecting hypocotyl tip and cotyledonary tip was measured.

As Table 11 shows, the growth after 24 hr is already sufficient to demonstrate that glutamine stimulates growth more than asparagine although asparagine stimulates also. Especially in the glutamine series the correlation between growth and initial length is again good.

TABLE 10

EFFECTS OF ASPARAGINE AND GLUTAMINE ON GROWTH OF EMBRYOS OF *DATURA STRAMONIUM*
Corrected estimates of length increment (y) of embryos at the mean initial length (x) of 365.5μ cultured for 48 hr in basal medium (control) and in media containing asparagine or glutamine.

The regression coefficient denotes the regression of length increment on initial length

Treat- ment No.	Treatment	No. of Observa- tions	Regression Coefficient	y in μ at $x = 365.7 \mu$	Treatments Differing Significantly from the Particular Treatment
1	Control	18	0.659	355.1	$1 < 2, 3$
2	Asparagine, 400 mg/l	16	0.659	618.8	$1 < 2 < 3$
3	Glutamine, 400 mg/l	15	0.659	924.9	$1, 2 < 3$

(h) *Hordeum vulgare*

H. vulgare was chosen as a representative of the Gramineae. The material was obtained from a single plant which, 2 months earlier, had been raised by explanting the immature embryo on an agar medium.

TABLE 11

EFFECTS OF ASPARAGINE AND GLUTAMINE ON GROWTH OF EMBRYOS OF *CHENOPODIUM ALBUM*
Corrected estimates of length increments (y) of embryos at the mean initial length (x) of 478.7μ cultured for 24 hr in basal medium (control) and in media containing asparagine or glutamine.

The regression coefficient denotes the regression of length increment on initial length

Treat- ment No.	Treatment	No. of Observa- tions	Regression Coefficient	y in μ at $x = 478.7 \mu$	Treatments Differing Significantly from the Particular Treatment
1	Control	13	0.163	188.2	$1 < 2, 3$
2	Asparagine, 400 mg/l	10	0.486	236.8	$1 < 2 < 3$
3	Glutamine, 400 mg/l	10	0.659	376.3	$1, 2 < 3$

To calculate an index for growth in this species, which is lacking a "torpedo" stage, the long axes of the scutella were measured. Measurement *in situ*, i.e. in the culture drop, was not possible in all cases, as the embryos adhere strongly to menisci. These circumstances account for high variability, and, in this species alone, statistical

treatment did not result in a significant difference between the asparagine and control series. Glutamine, however, proved significantly stimulating (see Table 12), and superior to asparagine.

The beneficial effect of asparagine on the growth of immature embryos of another gramineous plant, viz. corn, has been demonstrated by Haagen Smit, Siu, and Wilson (1945). It is quite certain now that addition of glutamine instead of asparagine will result in more vigorous growth.

TABLE 12

EFFECTS OF ASPARAGINE AND GLUTAMINE ON GROWTH OF EMBRYOS OF *HORDEUM VULGARE*
Corrected estimates of length increment (y) of embryos at the mean initial length (x) of 834.6μ cultured for 72 hr in basal medium (control) and in media containing asparagine or glutamine. The regression coefficient denotes the regression of length increment on initial length

Treat- ment No.	Treatment	No. of Observa- tions	Regression Coefficient	y in μ at $x = 834.6 \mu$	Treatments Differing Significantly from the Particular Treatment
1	Control	7	0.431	417.3	$1 < 3$
2	Asparagine, 400 mg/l	6	0.431	527.4	$2 < 3$
3	Glutamine, 400 mg/l	5	0.431	727.1	$1, 2 < 3$

(i) *Allium cepa*

A. cepa (Liliaceae) produces torpedo-shaped embryos; the one cylindrical cotyledon occupies the major part in the length. The results of one experiment are shown in Table 13. The three treatments are significantly different from each other. The glutamine series again does best, then the asparagine series.

TABLE 13

EFFECTS OF ASPARAGINE AND GLUTAMINE ON GROWTH OF EMBRYOS OF *ALLIUM CEPA*
Corrected estimates of length increments (y) of embryos at the mean initial length (x) of 750.1μ cultured for 48 hr in basal medium (control) and in media containing asparagine or glutamine.
The regression coefficient denotes the regression of length increment on initial length

Treat- ment No.	Treatment	No. of Observa- tions	Regression Coefficient	y in μ at $x = 750.1 \mu$	Treatments Differing Significantly from the Particular Treatment
1	Control	9	0.410	335.4	$1 < 2, 3$
2	Asparagine, 400 mg/l	9	0.371	492.8	$1 < 2 < 3$
3	Glutamine, 400 mg/l	9	1.260	846.1	$1, 2 < 3$

IV. DISCUSSION

The general outcome of the experiments may be summarized by means of a histogram (Fig. 4) which shows the general beneficial effect of glutamine and asparagine when applied to the medium of young embryos *in vitro* at a concentration of

400 mg/l. Within eight orders of angiosperms no exception to this rule is found for glutamine; for asparagine exceptions have been found in certain members of the Rhoeadales.

Further, glutamine is shown to enhance embryonic growth considerably more than asparagine in all cases. This result is considered in excellent agreement with recent biochemical literature which ascribes to glutamic acid and glutamine an

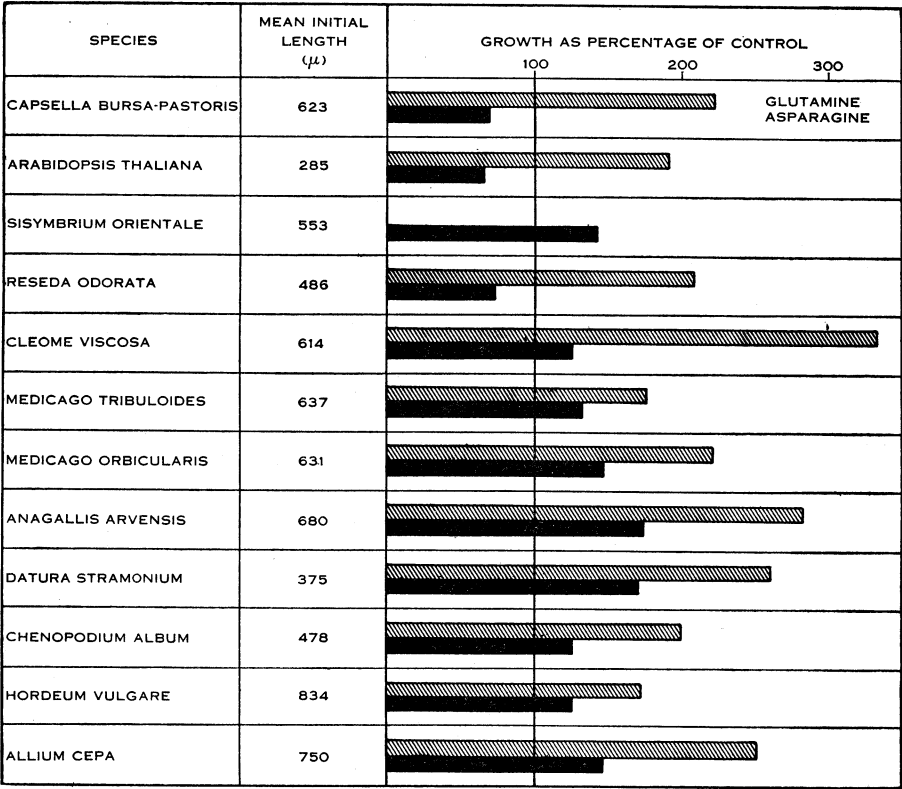


Fig. 4.—Histogram summarizing the effects of glutamine (400 mg/l) and asparagine (400 mg/l) on embryos of 12 species expressed as percentage of the growth in the control. The comparisons are made for 48 hr of culture, except for *Chenopodium album* (24 hr) and *Hordeum vulgare* (72 hr).

important primary role in plant nitrogen metabolism, and considers accumulation of asparagine rather the result of catabolic processes (Steward and Thompson 1954). This also holds for the Leguminosae (Meiss 1952); our results on the medics show that in this family also glutamine stimulates growth more than asparagine.

Glutamine, however, is not claimed to be superior to all amino acid mixtures, though this seems to be the case for “torpedoes” in *C. bursa-pastoris* (Rijven 1955).

Asparagine-induced inhibition of embryo growth can no longer be considered unique to *C. bursa-pastoris*. On the other hand, it was not possible to link the phenomenon as a taxonomic feature with Cruciferae or Rhoeadales though it has been

observed so far only in this order. It certainly does not occur in *Chenopodium album* which represents another one of Schulze's "glutamine plants" (Chibnall 1939).

It is interesting to note the enormous contrast between *C. bursa-pastoris* and *Medicago orbicularis*. In the latter, asparagine stimulates progressively up to 2000 mg/l while in the former it becomes inhibitory at concentrations above 50 mg/l.

In all those cases where asparagine stimulates growth, it does not interfere with glutamine utilization. This is brought out by experiments on *M. orbicularis*, *Anagallis arvensis*, and *Datura stramonium*. On the other hand, in *Arabidopsis thaliana*, as in *C. bursa-pastoris*, asparagine reduced the embryonic growth in a medium which also contained glutamine.

In a previous attempt to explain the asparagine-induced inhibition in *C. bursa-pastoris*, it was suggested that asparagine may block competitively an enzyme system which turns glutamine-N into protein-N (Rijven 1955). In this connection it was thought that specific transpeptidases or transaminases or both might be involved. For this hypothesis two main arguments could be advanced: first, the compounds are homologues and affect the growth in opposite ways, and second, glutamine reverses asparagine-induced inhibition while asparagine diminishes the effect of glutamine in concentrations of the same order of magnitude.

The hypothesis that glutamine-induced growth may be controlled by a specific enzymatic reaction is not discordant with the data presented in Table 1. On these data a test was made to check whether the growth values found do fit the Michaelis-Menten formula, which describes the rate of an enzyme reaction as a function of the concentration of its substrate

$$v = \frac{Vc}{c + K_m}, \quad \dots\dots\dots(2)$$

where v = reaction rate,
 c = concentration of substrate,
 $V = v$ at $c = \infty$,

and

$$K_m = c \text{ at } v = V/2.$$

As growth is found in the embryo culture in the control series, i.e. when glutamine is excluded from the medium, a displacement of the ordinate was assumed, whereby for embryo growth the formula becomes

$$y = \frac{Vc' + Vd}{c' + K_m + d}, \quad \dots\dots\dots(3)$$

where y = growth value in μ at x (= mean of initial length in 48 hr),
 c' = concentration of glutamine in mg/l,
 V = growth value at $c' = \infty$ in 48 hr,
 $K_m = c'$ at $y = V/2$,

and

d = displacement of ordinate in mg/l.

For simplicity the function tested was

$$y = \frac{Vc' + e}{c' + f} \dots \dots \dots (4)$$

Estimation of the parameters of this function, with the six entries of Table 1 fitting by the least squares method, yielded

$$V = 884.1 \mu,$$

$$e = 44116.59,$$

and

$$f = 131.4.$$

According to a χ^2 test the fit of the entries to equation (4) is quite satisfactory ($\chi^2 = 4.8888$, d.f. = 3, $0.25 > P > 0.10$). Thus for equation (3) it follows:

$$V = 884.1 \mu \text{ in 48 hr,}$$

$$K_m = 81.5 \text{ mg/l (= 0.56 mM),}$$

and

$$d = 49.9 \text{ mg/l (= 0.34 mM).}$$

The interpretation of the displacement d may only be guessed but it surely indicates the extent to which stored nitrogen is made available for residual growth.

The justification of the above mathematical treatment is weakened by two arguments: first, measurement of growth in length may perhaps not be accepted as a true representation of living matter or protein; and second, as the regression coefficients of the growth values do not follow a regular pattern (compare Table 1) the application of the formula may be restricted to the mean initial length of the embryos in this particular experiment.

The tentative explanation of asparagine-induced inhibition has now been complicated by the finding that asparagine did not prove inhibitory through all ranges of concentrations. Low concentrations of 10 mg/l proved slightly growth-promoting, and therefore it must be concluded that some enzyme system for the utilization of the asparagine-N must be present.

Higher concentrations of asparagine inhibit in the absence of glutamine, i.e. when compared with a control which does not contain nitrogen. Previously it was assumed that stored nitrogen for residual growth was made available via glutamine and that external asparagine reduced this residual growth. This may still hold if the asparagine enzyme system contributes so little in the synthetic direction that a large surplus of external asparagine blocks the main glutamine pathway thus effecting an overall growth depression. This is in line with the observation that the growth promotion with relatively low asparagine concentrations is very small indeed.

V. ACKNOWLEDGMENTS

I would like to express thanks to Mr. G. A. McIntyre and Mr. G. Dudzinski, Division of Mathematical Statistics, C.S.I.R.O., for statistical analysis of the experimental results, and to Mr. J. A. Zwar for help with the English text.

I would also like to acknowledge the help of my colleagues in providing some of the material, and of Messrs. L. G. Lee, Director, Kimberley Research Station, C.S.I.R.O., W. Arndt, Officer-in-Charge, Katherine Research Station, C.S.I.R.O., and G. Chippendale, Botanist, Animal Industry Division, Alice Springs, for the seed of *Cleome viscosa*.

The technical assistance of Mrs. S. Sapiets is gratefully mentioned.

VI. REFERENCES

- CHIBNALL, A. C. (1939).—"Protein Metabolism of Plants." 1st Ed. (Yale Univ. Press: New Haven.)
- HAAGEN SMIT, A. J., SIU, R., and WILSON, G. (1945).—*Science* **101**: 234.
- LINDERSTRØM LANG, K., and HOLTER, H. (1941).—Die enzymatische Histochemie. In "Die Methoden der Ferment forschung." Bd. 1. p. 1132. (Ed. E. Bamann and K. Myrbäck.) (J. Springer: Berlin.)
- MEISS, A. N. (1952).—Bull. Conn. Agric. Exp. Sta. No. 553.
- PARIS, D., RIETSEMA, J., SATINA, S., and BLAKESLEE, A. F. (1953).—*Proc. Nat. Acad. Sci., Wash.* **39**: 1205.
- RAPPAPORT, J. (1954).—*Bot. Rev.* **20**: 201.
- RIETSEMA, J., SATINA, S., and BLAKESLEE, A. F. (1953).—*Amer. J. Bot.* **40**: 538.
- RIJVEN, A. H. G. C. (1952).—*Acta Bot. Neerl.* **1**: 159.
- RIJVEN, A. H. G. C. (1955).—*Proc. Acad. Sci. Amst.* (series C) **50**: 368.
- STEWART, F. C., and THOMPSON, J. F. (1954).—Proteins and protein metabolism in plants. In "The Proteins." Vol. 2. Pt. A. Ch. 19. p. 513. (Ed. H. Neurath and K. Bailey.) (Academic Press Inc.: New York.)