Biosynthesis of 2,4-Diaminobutyric Acid from L-[^{3}H]Homoserine and DL-[1-^{14}C]Aspartic Acid in *Lathyrus sylvestris* W.*

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ABSTRACT: Biosynthesis of 2,4-diaminobutyric acid in seedlings of *Lathyrus sylvestris* W. has been investigated. L-[^{3}H]Homoserine was administered to the cut seedlings which resulted in effective incorporation of radioactivity into free 2,4-diaminobutyric acid. Administration of DL-[1-^{14}C]aspartic acid also resulted in significant labeling of 2,4-diaminobutyric acid, as well as many other substances. Free homoserine labeled by the [1-^{14}C]aspartic acid had a higher specific activity than the 2,4-diaminobutyric acid and showed essentially the same distribution of radioactivity between C-1 and the rest of the molecule. It seems likely that 2,4-diaminobutyric acid is synthesized from both homoserine and aspartic acid and that there is a close relationship in the pathways for the formation of homoserine and 2,4-diaminobutyric acid from aspartic acid.

2,4-Diaminobutyric acid is a comparatively new member of the group of naturally occurring diamino-acids that include lysine, ornithine, and 2,3-diaminopropionic acid. It does not appear to be present in protein and until recently was considered chiefly as an important constituent of a large number of naturally occurring peptide antibiotics that include polypeptin, comirin, and members of the polymyxin, colistin, and circulin families (Hausmann and Craig, 1952; Forsyth, 1955; Jones, 1949; Suzuki *et al.*, 1963; Koffler, 1959). Recently, in an investigation of various seeds of certain legumes of interest in connection with the neurological disease lathyrism, L-2,4-diaminobutyric acid was isolated and identified as the chief toxic principle of *Lathyrus latifolius* (perennial sweet pea) (Ressler *et al.*, 1961). The free amino acid is now known to occur frequently in significant amount in seeds of species of *Lathyrus* (Bell, 1962, 1964). A number of these contain, as well, smaller amounts in a form tentatively identified as the γ-oxalyl derivative and appear to have still another acidic derivative, as yet unidentified (Bell, 1964). 2,4-Diaminobutyric acid in small or trace amount is present in seeds of a large number of other species (Zacharius *et al.*, 1955; Fowden and Bryant, 1958; Van Etten and Miller, 1963), especially those belonging to the *Leguminosae* and the *Cruciferae* families (Van Etten and Miller, 1963). A monoacetyl 2,4-diaminobutyric acid is found in the latex of *Euphorbia pulchérrima*, Willd. (poinsettia) (Liss, 1962). 2,4-Diaminobutyric acid is a major component of the mucopeptide of the cell wall of the plant pathogen *Corynebacterium triticii* 471 (Perkins and Cummins, 1964). It has been detected also in a lower animal, *Arion empiricorum* (road snail) (Ackermann and Menssen, 1960).

The toxic properties of 2,4-diaminobutyric acid and the increasing evidence for a wider distribution of it in nature than previously suspected have interested us in studying the biosynthesis of this amino acid. At the start of this investigation, no information was available on this subject.[1] Seedlings of *L. sylvestris* W. were chosen for this study in view of their high content (2.6%) of free 2,4-diaminobutyric acid (Ressler, 1964). Experiments were planned with the possibility in mind that the pathway for the biosynthesis of 2,4-diaminobutyric acid might be similar, in general, to one of those established for its higher homologs ornithine and lysine. These amino acids are considered to be related biosynthetically in certain systems to the corresponding dicarboxylic amino acids. In the rat, certain fungi, and bacteria, ornithine appears to be formed from glutamic acid, although it is not clear that the intermediates are identical for each group (Stetten, 1954; Meister, 1965a). In certain fungi lysine is considered to be formed from α-aminoadipic acid, whereas in bacteria and some green organisms it is formed from α,ϵ-diaminopimelic acid (Meister, 1965b).

In the present investigation DL-[1-^{14}C]aspartic acid was tested as a possible precursor of 2,4-diaminobutyric acid. Homoserine is thought to be formed from aspartic acid in pea seedlings (Naylor *et al.*, 1958; Sasaoka, 1961) and is present in high concentration (1.3–1.6%)

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[1] In a study of the metabolism of β-cyanoalanine in seedlings of *L. sylvestris* W., it was found that although L-[4-^{14}C]-β-cyanoalanine was incorporated into asparagine to the extent of 33.5% (Ressler *et al.*, 1963), its incorporation into 2,4-diaminobutyric acid was only 0.27% (Y. H. Giza and C. Ressler, 1962, unpublished data). Reduction of β-cyanoalanine, a pathway which had been considered earlier (Ressler *et al.*, 1961), therefore seemed unlikely as a significant direct route to 2,4-diaminobutyric acid in this plant. Recently, Tschiersch (1964) reported, on the basis of autoradiographic evidence, that H^{14}CN administration to seedlings of *Vicia sativa* (common vetch) results in labeled 2,4-diaminobutyric acid; he considered this conversion to be mediated by β-cyanoalanine.

in seedlings of *L. sylvestris*. Homoserine, labeled with tritium, was therefore also given to the seedlings. The results suggest that 2,4-diaminobutyric acid can be formed from both homoserine and aspartic acid. Homoserine, particularly, served as an efficient precursor. Degradation experiments showed that, after administration of the DL-[1-^{14}C]aspartic acid, both 2,4-diaminobutyric acid and tissue homoserine were preferentially labeled in C-1, and to a similar degree. This suggests a close relationship in this plant in the pathways for the formation of the diamino acid and homoserine from aspartic acid.

Experimental Procedure

Materials. D. D. Dolan (New York State Agricultural Experiment Station, Geneva, N. Y.) generously provided seeds of *L. sylvestris* W.; E. A. Bell (King's College, London), a reference sample of oxalyl-2,4-diaminobutyric acid. L-[*g*-^{3}H]Homoserine was purchased from Calbiochem, Los Angeles, Calif., and DL-[1-^{14}C]aspartic acid from NiChem, Inc., Bethesda, Md.

Conditions of Culture and Experiments. Seeds were washed with water and germinated in Petri dishes in the dark. After 2 weeks the seedlings were transferred to gravel and were illuminated 10 hr daily. On alternate days they received nutrient salt solution (Waller and Henderson, 1961) and distilled water. At the end of 4 weeks in expt 1 and 3 weeks in expt 2, the seedlings were cut just above the place of emergence. A small further section was removed under water with a sharp razor blade. The cut end was dipped into the solution of the labeled amino acid in a conical-shaped container. Twenty seedlings were used in expt 1 with L-[^{3}H]homoserine dissolved in 0.6 ml of water; 24 seedlings from a separate batch were used in expt 2 with DL-[1-^{14}C]-aspartic acid dissolved in 1.95 ml of water. After several hours most of the tracer solution had been taken up, and a few milliliters of water was added. The seedlings were illuminated continuously for 9 hr and then removed, rinsed, and cut into small portions. The material was dried under vacuum over P_2O_5. The flasks were rinsed with water, and the remaining radioactivity was measured to allow calculation of the uptake of radioactivity.

Isolation and Determination of Amino Acids. Crude extracts were prepared from the dried seedlings as described earlier (Nigam and Ressler, 1964). Amino acids were determined by the chromatographic ninhydrin procedure with the Beckman-Spinco Model 120 automatic amino acid analyzer. Neutral and acidic amino acids were determined with the system recommended for physiological fluids: the 150-cm column at pH 3.25 and 30° with a change to pH 4.25 and 50° at 11–13 hr (Spackman *et al.*, 1958). Basic amino acids were determined on the 50-cm column at pH 4.26 and 50° (Ressler and Kashelikar, 1966). Asparagine was determined by treatment of the seedling extract with guinea pig serum asparaginase and was calculated from the resultant increase in aspartic acid which was determined on the amino acid analyzer. To the sample of the extract that represented 9.85 mg of seedlings in 50 μl of water were added 175 μl of water, 475 μl of 0.01 M sodium borate buffer, pH 8.5, and 175 μl of the asparaginase preparation (Meister, 1955). The mixture was incubated at 38° for 2 hr and adjusted to pH 2.2; one-fourth of it was analyzed.

Chromatography was carried out on Whatman No. 1 or No. 3MM paper with system 1: *n*-butyl alcohol–acetic acid–water (4:1:5), upper phase; system 2: 75% phenol; system 3: *n*-butyl alcohol–pyridine–water (1:1:1); system 4: *n*-butyl alcohol–pyridine–water–acetic acid (1:1:1:1).

After isolation by paper or column chromatography amino acids were dissolved in water and analyzed on the amino acid analyzer to determine their content and purity. Table I gives the amount present in the seedlings in the free state. The chromatographically homogeneous amino acids were then diluted with carrier and crystallized from water–ethanol to constant specific activity.

Isolation of Free 2,4-Diaminobutyric Acid. Material in the crude extracts was subjected to electrophoresis as a band on Whatman 3MM paper in pyridinium acetate buffer, pH 5.7, for 3 hr at 9 v/cm. Bands of basic, neutral, and acidic material were located with ninhydrin, cut out, and eluted with water. In expt 1 the basic material was chromatographed on paper in system 2. Material of R_F 0.2 corresponding to 2,4-diaminobutyric acid was eluted and rechromatographed on paper in system 1 in which it had R_F 0.07. In expt 2 the basic material was chromatographed directly in system 1.

Isolation of Homoserine, Threonine, and Asparagine. In expt 1 the neutral material obtained on electrophoresis of material extracted from 118 mg of seedlings was chromatographed as a band on paper in system 2. The highly radioactive ninhydrin-positive band, R_F 0.59, corresponding to homoserine was eluted. In expt 2, homoserine was isolated by chromatography on the amino acid analyzer. For this it was desirable to remove first the large amount of labeled asparagine present which emerges just before homoserine, elution volumes 187 and 200 ml, respectively. The neutral material corresponding to 315 mg of the seedlings was therefore treated with asparaginase as described for the determination of asparagine. The incubation mixture was then adjusted to pH 4.0 with acetic acid and applied to a column of Dowex 1-X4 resin (acetate cycle, 1.4 × 23 cm). The column was washed with 100 ml of water. Elution was continued with 0.5 N acetic acid (Hirs *et al.*, 1954), and the aspartic acid formed from asparagine was recovered and crystallized; wt 14.9 mg. The water wash containing the neutral amino acids was concentrated and chromatographed on the 150-cm column of the amino acid analyzer at 30°. The effluent was collected in 2-ml fractions. Fractions containing homoserine were pooled, concentrated, and applied to a column of Dowex 50-X4 (H^+ cycle, 1.4 × 10 cm) to remove buffer salts. The column was washed with three column volumes of deionized water followed by 3 N ammonia.

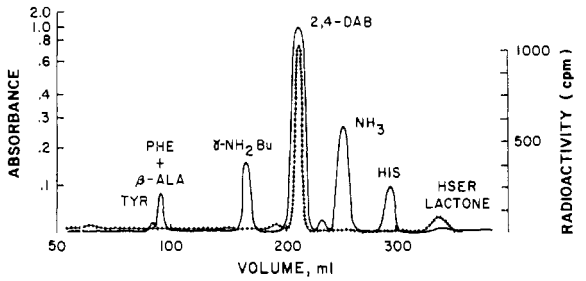


FIGURE 1: Chromatogram of a crude extract of a 9.85-mg sample of seedlings of *L. sylvestris* W. that had received tritiated L-homoserine, 2.55×10^7 cpm. ——, ninhydrin-positive components; ····, radioactivity. The 50-cm column of the amino acid analyzer was used at pH 4.26 and 50° (see expt 1 in "Conditions of Culture and Experiments," and "Isolation and Determination of Amino Acids").

Elution with ammonia was continued until 5 ml after the effluent became alkaline. The ammonia eluate was evaporated to dryness. Evaporation was repeated with additions of small amounts of water.

For isolation of threonine a portion of the neutral fraction in expt 1 corresponding to 59 mg of the seedlings was similarly chromatographed on the 150-cm column of the amino acid analyzer. Since the amount of threonine was small, it was diluted with 11.7 mg of carrier before desalting.

Isolation of Aspartic Acid, Glutamic Acid, α-Aminoadipic Acid, and Bound 2,4-Diaminobutyric Acid. In expt 1, the acidic fraction obtained by electrophoresis of material extracted from 118 mg of seedlings was chromatographed on a column of Dowex 1-X4 resin (acetate cycle, 100–200 mesh), 0.6 × 55 cm (Hirs *et al.*, 1954). Fractions containing the acidic amino acids were located by ninhydrin and radioactivity determinations; elution volumes were 140, 45, and 30 ml, respectively.

In expt 2, similarly obtained acidic material from 315 mg of seedlings was chromatographed on the column of Dowex 1-X4 resin. Elution was carried out with 200 ml of 0.5 N acetic acid followed by 100 ml of 0.5 N pyridinium acetate buffer, pH 4.1, then by 300 ml of 1 N buffer of the same pH. Ninhydrin-positive material at 360 ml, which was weakly radioactive, was chromatographed on paper in system 2. It afforded a major band of R_F 0.24. Its mobility in this system, in system 4 (R_F 0.20), and on paper electrophoresis at pH 5.7 agreed with that of a reference sample of oxalyl-2,4-diaminobutyric acid (Bell, 1964). The material was eluted and hydrolyzed in 6 N HCl at 110° for 18 hr. The liberated 2,4-diaminobutyric acid was isolated by paper electrophoresis at pH 5.7.

Degradation of 2,4-Diaminobutyric Acid and Homoserine. Radioactivity in C-1 of the isolated 2,4-diaminobutyric acid and homoserine was determined by decarboxylation with ninhydrin (Greenberg and Rothstein, 1957). The liberated CO_2 was led with a stream of N_2 into a trap containing 2 ml of carbonate-free 1 N NaOH. The contents of the trap were plated as a single pad of $BaCO_3$ and counted. Decarboxylations, run in duplicate, of the two nonisotopic amino acids resulted in quantitative yields of $BaCO_3$.

2,4-Diaminobutyric acid was degraded to β-alanine essentially according to the procedure used by Strassman and Weinhouse (1953) to convert lysine to δ-aminovaleric acid, modified with respect to the isolation of the product. After 24 hr the reaction mixture was centrifuged, the supernatant was removed, and the residue was extracted with 10 ml of water. The combined supernatants were desalted on a column of Dowex 50-X8 resin (H^+ cycle), 13 × 1 cm, as described for isolation of threonine. Amino acid analysis of the desalted product showed β-alanine in 41% yield, unidentified material eluted near ammonia, and no detectable 2,4-diaminobutyric acid. The β-alanine was then chromatographed on paper in system 1, R_F 0.2, and the eluted material was crystallized twice as the hydrochloride from alcohol–ether. The β-alanine was homogeneous on the analyzer and afforded a single radioactive and ninhydrin-reactive spot on paper chromatography in systems 1 and 3. Specific activities of the degradation products are given in Table II.

Determination of Radioactivity. C-14 was determined as described earlier (Nigam and Ressler, 1964). Tritium was counted in a Nuclear-Chicago 722 liquid scintillation system. Samples of the aqueous solution were dissolved in 15 ml of scintillation mixture (Bray, 1960). Correction for quenching was made by adding a known amount of tritiated toluene and recounting the sample (Francis *et al.*, 1959). Crude extracts were scanned for radioactivity by routing the effluent from the 150- and 50-cm resin columns of the amino acid analyzer through a 2-ml flow cell with a Nuclear-Chicago 6770 scintillation-flow counter adapter used in conjunction with the liquid scintillation system.

Results and Discussion

Table I gives the results of experiments in which L-[^{3}H]homoserine (expt 1) and DL-[1-^{14}C]aspartic acid (expt 2) were given to the seedlings of *L. sylvestris* W. The findings in expt 1 show that [^{3}H]homoserine can serve as an effective precursor of 2,4-diaminobutyric acid; more than 6% of its radioactivity was incorporated into the free 2,4-diaminobutyric acid. Figure 1 shows the relative distribution of radioactivity in a chromatogram of some of the basic amino acids present in the free state in the tissues after administration of [^{3}H]homoserine. The effective labeling of 2,4-diaminobutyric acid is apparent. From this chromatogram and that of the neutral and acidic amino acids, it was clear that homoserine, 2,4-diaminobutyric acid, and threonine have a large proportion of the label.

The observed labeling of threonine is in general agreement with the precursor relationship of homoserine to threonine established in *Escherichia coli* and certain fungi (Meister, 1965c). As can be seen from Table I, the specific activity of threonine (1.86×10^5 cpm/μmole) is seven times that of 2,4-diaminobutyric acid (2.74 ×

TABLE I: Biosynthesis of 2,4-Diaminobutyric Acid in Seedlings of *L. sylvestris* W. from L-[^{3}H]Homoserine and DL-[1-^{14}C]Aspartic Acid.

Expt[a]	Precursor: Administered	Precursor: Uptake[b] (cpm × 10^{-6}) (*A*)	Free Amino Acid Isolated from Tissue	Tissue Content (μmole) (*B*)	Sp Act. (cpm × 10^{-3}/μmole) (*C*)	Total Act. (cpm × 10^{-4}) (*BC*)	% Incorpd (*BC*/*A* ×100)
1	L-[^{3}H]Homoserine, 2.55 × 10^7 cpm, 1.04 μmoles	23.4	2,4-Diaminobutyric acid	54.3	27.4	149	6.36
			Threonine	1.4	186	26.0	1.11
			Aspartic acid	2.0	5.39	1.08	0.05
			Glutamic acid	1.0	10.1	1.01	0.04
			α-Aminoadipic acid	1.75	7.0	1.23	0.05
			Homoserine	23.9	186	445	19.0
2	DL-[1-^{14}C]Aspartic acid, 3.77 × 10^7 cpm, 48.4 μmoles	36.7	2,4-Diaminobutyric acid	79.4	4.27	33.9	0.94
			Homoserine	56.8	17.2	97.7	2.66
			Asparagine	182	3.53	64.2	1.75

[a] In expt 1 and 2, the dry weight of seedlings was 213 and 414 mg, respectively. Concentrations of 2,4-diaminobutyric acid and homoserine in expt 1 were 2.95 and 1.34%; in expt 2, 2.28 and 1.63%. Radioactivity incorporated into the seedlings in expt 2 was 63% of the uptake, *A*. [b] Uptake represents the difference between the amount of radioactivity administered and that left in the medium at the end of the experiment, and was 92 and 97% of the administered activity.

10^4 cpm/μmole). The total amount of 2,4-diaminobutyric acid (54.3 μmoles) in the seedlings is 39 times that of threonine (1.4 μmoles). If one attempts to consider relative dilutions of radioactivity resulting from the difference in the size of the amino acid pools, the specific activity of 2,4-diaminobutyric acid is multiplied by 39 and it then becomes higher than that of threonine. If certain additional factors affecting specific activity are not considered, *i.e.*, relative rates of formation and utilization of threonine and 2,4-diaminobutyric acid, then homoserine would seem to be at least as good a precursor of 2,4-diaminobutyric acid as it is of threonine. Aspartic acid was also labeled by the administered [^{3}H]homoserine. Its specific activity, however, was only about one-fifth that of the 2,4-diaminobutyric acid. This makes it seem unlikely that aspartic acid is a significant intermediate between homoserine and 2,4-diaminobutyric acid, although the possibility that this route occurs to some extent cannot be ruled out.

The administered DL-[1-^{14}C]aspartic acid was also significantly converted to free 2,4-diaminobutyric acid. Specific activity of the latter was 4.27 × 10^3 cpm/μmole; total incorporation of radioactivity was nearly 1%. 2,4-Diaminobutyric acid isolated from a derivative with the chromatographic behavior of oxalyl-2,4-diaminobutyric acid (Bell, 1964), in contrast, had little activity; its specific activity was only 107 cpm/μmole.

Tissue homoserine was efficiently labeled. Its specific activity and the total activity incorporated into it were higher than the corresponding values for 2,4-diaminobutyric acid. Under the conditions chosen, homoserine appears to be one of the major amino acid metabolites of aspartic acid. This reaction accounted for an even larger proportion of the metabolism of aspartic acid than its incorporation into asparagine. Naylor *et al.* (1958) reported similar effective labeling of homoserine by [^{14}C]aspartic acid in roots of pea plants. Numerous other substances also became labeled; considerable amounts of radioactivity were present on amino acid analysis in the chromatographic regions corresponding to alanine, γ-aminobutyric acid, homoserine lactone, and threonine, as well as aspartic acid. These substances were not isolated or further investigated in view of the known high metabolic activity of aspartic acid and the apparent similarity of many of these results to the findings of Naylor *et al.* (1958). The identity of highly radioactive, ninhydrin-positive material eluted in the acidic region near the column breakthrough volume is under investigation.

Table II shows the distribution of radioactivity in the 2,4-diaminobutyric acid and homoserine formed from DL-[1-^{14}C]aspartic acid, the results of degradation experiments. Decarboxylation with ninhydrin showed that C-1 of the formed 2,4-diaminobutyric acid had 58%, and C-1 of the formed homoserine 65% of the total radioactivity in the respective molecules. Direct examination of C-2, -3, and -4 of the 2,4-diaminobutyric acid molecule, in the form of β-alanine obtained from it by permanganate oxidation, showed, in good agreement, 39% of the activity of the 2,4-diaminobutyric acid to be present in this portion of the molecule. The 35–42% randomization of C-1, which apparently took place in the conversion of aspartic acid to 2,4-diaminobutyric acid and homoserine, is not surprising. Aspartic

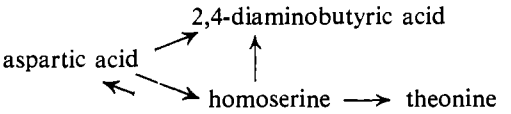
TABLE II: Distribution of Radioactivity in 2,4-Diaminobutyric Acid and Homoserine Formed from DL-[1-^{14}C]Aspartic Acid by Seedlings of *L. sylvestris* W.

Degradation					Fragment					
Compd, Sp Act. (cpm/ μmole) (*A*)	Procedure	Carrier Sample (mg)	Carrier Added (mg)	DF[a]	Examined	Wt (mg)	Act. (cpm)	Spc Act.[b] (cpm/ μmole) (*B*)	% Distribn of Label [^{1}C] (*B*/*A*) × 100	% Distribn of Label [^{2,3,4}C] (*B*/*A*) × 100
L-2,4-Diamino-butyric acid·HCl 721	Ninhydrin	1.19	11.35	10.54	$BaCO_3$ [^{1}C]	16.4	3,318	417	57.8	
	Permanganate	2.58	38.92	16.09	β-Alanine·HCl[c] [^{2,3,4}C]	1.01	141	280		38.8
L-Homoserine 2700	Ninhydrin	0.76	11.13	15.64	$BaCO_3$ [^{1}C]	19.4	10,958	1752	64.9	

[a] Dilution Factor. [b] Corrected for dilution. [c] Counted as $BaCO_3$.

acid administered to various other plants has been shown to be closely linked metabolically to the organic acids of the citric acid cycle (Naylor *et al.*, 1958). In their short-term experiments malic acid received the preponderance of the label from [^{14}C]aspartic acid. It was suggested that administered aspartic acid might enter the citric acid cycle after conversion to either oxaloacetate or fumarate, the formation of the latter being catalyzed possibly by aspartase (Naylor *et al.*, 1958). Loss of specificity of the label in aspartic acid is expected from some regeneration of oxaloacetate and aspartate from the symmetrical fumarate molecule. It is interesting that the degree of randomization of C-1 of aspartic acid observed in the present synthesis of 2,4-diaminobutyric acid and homoserine is somewhat similar to that observed by Delluva (1953), studying the biosynthesis of threonine from [3-^{14}C]aspartic acid in *E. coli* in which homoserine is now considered to be the intermediate (Meister, 1965c). There, the formed threonine had 70% of its activity in C-3.

The results of the present isotope experiments suggest that 2,4-diaminobutyric acid is formed from both homoserine and aspartic acid in seedlings of *L. sylvestris*. Homoserine seemed to be a better precursor than aspartic acid of 2,4-diaminobutyric acid. However, it should be noted that the two experiments were not carried out simultaneously and, although conditions were similar, the percentages of radioactivity incorporated into 2,4-diaminobutyric acid from the two amino acids probably should not be compared rigorously. Homoserine synthesis from aspartic acid also seems to be an efficient reaction in this plant. The relative specific activities of homoserine and 2,4-diaminobutyric acid after administration of DL-[^{14}C]aspartic acid and the resulting marked similarity in the formed homoserine and 2,4-diaminobutyric acid, with regard to the distribution of activity between C-1 and the rest of the respective molecules, are consistent with the possibility that homoserine could be an intermediate in the conversion of aspartic acid to 2,4-diaminobutyric acid. However, the data do not preclude the possibility of separate pathways for homoserine and aspartic acid to 2,4-diaminobutyric acid. The findings are in accord with the accompanying metabolic scheme.



Studies on the enzymic level have indicated that the synthesis of homoserine from aspartic acid in microorganisms and pea seedlings probably proceeds through formation of β-aspartyl phosphate followed by aspartic β-semialdehyde, with the latter being converted to homoserine through the mediation of homoserine dehydrogenase (Meister, 1965c). If aspartic β-semialdehyde could be converted to 2,4-diaminobutyric acid, perhaps by a transamination reaction, one could picture separate routes from aspartic acid and homoserine to 2,4-diaminobutyric acid, each proceeding through aspartic β-semialdehyde as a common intermediate. The intermediate steps in the conversion of aspartic acid and homoserine to 2,4-diaminobutyric acid and their requirements remain to be established.

Participation of homoserine in amino acid metabolism is considered at present to be limited to lower forms of life (Virtanen and Meittinen, 1953; Przybylska, 1963; Teas *et al.*, 1948; Fling and Horowitz, 1951). There is some evidence, however, indicating that homoserine can be formed also by mice and rats after administration of methionine or ethionine (Matsuo and Greenberg, 1955; Chatagner, 1964). It will be of interest to determine whether the biological conversion of homoserine and aspartic acid to the toxic 2,4-diaminobutyric acid could have significance in mammalian metabolism.

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References

Ackermann, D., and Menssen, H. G. (1960), *Z. Physiol. Chem. 318*, 212.
Bell, E. A. (1962), *Nature 193*, 1078.
Bell, E. A. (1964), *Nature 203*, 378.
Biserte, G., and Dautrevaux, M. (1957), *Compt. Rend. Soc. Biol. 151*, 1888.
Bray, G. A. (1960), *Anal. Biochem. 1*, 279.
Chatagner, F. (1964), *Nature 203*, 1177.
Delluva, A. M. (1953), *Arch. Biochem. Biophys. 45*, 443.
Fling, M., and Horowitz, N. H. (1951), *J. Biol. Chem. 190*, 277.
Forsyth, W. G. C. (1955), *Biochem. J. 59*, 500.
Fowden, L., and Bryant, M. (1958), *Biochem. J. 70*, 626.
Francis, G. E., Mulligan, W., and Wormall, A. (1959), *in* Isotopic Tracers, London, Athlone Press, p 238.
Fu, S.-C. J., Rao, K. R., Birnbaum, S. M., and Greenstein, J. P. (1952), *J. Biol. Chem. 199*, 207.
Greenberg, D. M., and Rothstein, M. (1957), *Methods Enzymol. 4*, 711.
Hausmann, W., and Craig, L. C. (1952), *J. Biol. Chem. 198*, 405.
Hirs, C. H. W., Moore, S., and Stein, W. H. (1954), *J. Am. Chem. Soc. 76*, 6063.
Jones, T. S. G. (1949), *Ann. N. Y. Acad. Sci. 51*, 909.
Koffler, H. (1959), *Science 130*, 1419.
Liss, I. (1962), *Phytochemistry 1*, 87.
Matsuo, Y., and Greenberg, D. M. (1955), *J. Biol. Chem. 215*, 547.
Meister, A. (1955), *Methods Enzymol. 2*, 383.
Meister, A. (1965), *in* Biochemistry of the Amino Acids, Vol. 2, New York, N. Y., Academic: (a) p 709, (b) p 928, (c) pp 674–678.
Naylor, A. W., Rabson, R., and Tolbert, N. E. (1958), *Physiol. Plantarum 11*, 537.
Nigam, S. N., and Ressler, C. (1964), *Biochim. Biophys. Acta 93*, 339.
Perkins, H. R., and Cummins, C. S. (1964), *Nature 201*, 1105.
Przybylska, J. (1963), *Ann. Rept. of Polish Acad. Sci.*, Institute of Plant Genetics, Poland.
Ressler, C. (1964), *Federation Proc. 23*, 1350.
Ressler, C., Giza, Y.-H., and Nigam, S. N. (1963), *J. Am. Chem. Soc. 85*, 2874.
Ressler, C., and Kashelikar, D. V. (1966), *J. Am. Chem. Soc. 88*, 2025.
Ressler, C., Redstone, P. A., and Erenberg, R. H. (1961), *Science 134*, 188.
Sasaoka, K. (1961), *Plant Cell Physiol. (Tokyo) 2*, 231.
Spackman, D. H., Stein, W. H., and Moore, S. (1958), *Anal. Chem. 30*, 1190.
Stetten, M. R. (1954), *in* Symposium on Amino Acid Metabolism, McElroy, W. D., and Glass, B., Ed., Baltimore, Md., Johns Hopkins, p 277.
Strassman, M., and Weinhouse, S. (1953), *J. Am. Chem. Soc. 75*, 1680.
Suzuki, T., Inouye, H., Fujikawa, K., and Suketa, Y. (1963), *J. Biochem. (Tokyo) 54*, 25; *Chem. Abstr. 60*, 12254*g*.
Teas, H. J., Horowitz, N. H., and Fling, M. (1948), *J. Biol. Chem. 172*, 651.
Tschiersch, B. (1964), *Phytochemistry 3*, 365.
Van Etten, C. H., and Miller, R. W. (1963), *Econ. Botany 17*, 107.
Virtanen, A. I., and Meittinen, J. K. (1953), *Biochim. Biophys. Acta 12*, 181.
Waller, G. R., and Henderson, L. M. (1961), *J. Biol. Chem. 236*, 1186.
Zacharius, R. M., Pollard, J. K., and Steward, F. C. (1955), *Ann. Acad. Sci. Fennicae A11 60*, 321.