plants, it was found that, with a nutrient level of 200 ppm of Na_2EDTA supplied to plants in nutrient solutions, the respiration rate of sunflower leaf tissue was only about 60 percent, the catalase activity was about 30 percent, and total fresh weight yield was about 54 percent of the respective values of leaf tissues from plants supplied with 5 ppm of Na_2EDTA. These results suggest that there is a competition between EDTA and the enzymes in the plant for metals essential for enzyme activity.

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Enzymatic Transfer of Alpha-Amino Groups*

Alton Meister

National Cancer Institute, National Institutes of Health, Bethesda, Maryland

THE first step in the metabolism of many amino acids in animals, plants, and microorganisms is separation of the α-amino group from the amino acid carbon chain, resulting in the formation of an α-keto acid. Reversal of this reaction—that is, conversion of a keto acid to an amino acid—is frequently the final step in the biosynthesis of an amino acid.

With the demonstration of enzymatic transamination by Braunstein and Kritsmann (*1*), it became apparent that interconversion between certain amino and keto acids took place by an intermolecular transfer of an amino group from an amino acid to a keto acid by a mechanism not involving the intermediate participation of ammonia. Although the enzymatic transamination reaction was first described in 1937, it has been only recently that the extent and significance of amino group transfer reactions in the anabolism and catabolism of the amino acids has been recognized.

Several years ago, it was generally believed that three amino acids (alanine, aspartic acid, and glutamic acid) were the major, if not the sole participants in transamination (*2, 3*). However, the rapid incorporation of administered amino acid nitrogen into almost all the amino acids in animals (*4*) and the ability of the α-keto analogs of certain essential amino acids to substitute for these in supporting the growth of animals and microorganisms made highly probable the occurrence of reactions involving the exchange of amino groups of many other amino acids.

The major changes that have taken place in our understanding of the role of transamination in the biosythesis and degradation of the amino acids have been due to large measure to the development of new techniques of amino acid and keto acid preparation, identification, and quantitation. It now appears that virtually all the naturally occurring amino acids participate in transamination reactions, and that these reactions are catalyzed by a number of separate transaminase enzyme systems. Finally, as a result of recent work, the function of two phosphorylated derivatives of vitamin B_6, pyridoxal phosphate and pyridoxamine phosphate, as coenzymes for transaminase has become more firmly established.

This article considers some of the results obtained in the course of studies on transamination carried out in our laboratory (*5*). It was recognized at the start of these investigations that a comprehensive study would require pure preparations of the optical isomers and α-keto analogs of a wide variety of amino acids. The enzymatic methods of resolution of racemic amino acids developed by Greenstein and collaborators (*6*) have made possible the preparation of the D- and L-isomers of all the naturally occurring (and a number of other) amino acids in good yield and in a state of high optical purity (*7*). I was fortunate in being associated with some of these studies and to have been the recipient of generous amounts of these optically pure amino acids, which were essential for the present

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work. At this time, only some of the α-keto analogs of the naturally occurring amino acids were known and could be prepared by methods described in the literature. It therefore became necessary to develop procedures for the preparation of other α-keto acids.

A general method was devised whereby amino acid isomers were oxidized by optically specific oxidases to the corresponding α-keto acids, which were purified by ion-exchange chromatography (or distillation), and crystallized as free acids or salts. In this manner, more than 30 α-keto acids have been prepared (*8, 9*) including those corresponding to arginine, citrulline, asparagine, glutamine, lysine, and ornithine. Through this work, preparation and study of the optical isomers of the α-keto analogs of isoleucine became possible. The adjacent positions of the carbonyl and asymmetric carbon atoms in this molecule provided a substrate advantageous for the consideration of certain aspects of enolization and α,β-unsaturation in transamination and other enzymatic reactions.

The present studies have revealed the function of glutamine and asparagine as amino group donors in transamination reactions leading to the formation of many of the naturally occurring amino acids. The α-keto acid-ω-amides corresponding to glutamine and asparagine are formed and hydrolyzed in the course of these reactions, which represent new pathways in the metabolism of these amino acid amides. The formation of asparagine from α-ketosuccinamate has been demonstrated, suggesting a possible pathway of asparagine biosynthesis.

Further evidence of the multiplicity of transaminase systems has come to light from investigations of a mutant bacterium in which the genetic modification is associated with loss of a transaminase specific for isoleucine and valine. Despite the wide scope of transamination, it is evident that each of the enzymes that catalyze these reactions possesses specific substrate requirements. For example, an enzyme fraction obtained from a bacterial organism catalyzes transamination only between glutamate, aspartate, phenylalanine, tyrosine, tryptophan, and their α-keto analogs, while another enzyme obtained from the same cell, catalyzes reactions only between valine and alanine or α-aminobutyric acid.

A relationship between vitamin B_6 and transamination was suggested and experimentally established in earlier studies, and this vitamin, in the form of pyridoxal phosphate, was considered to be the coenzyme for transaminase. Our investigations have been concerned in part with the role of the phosphorylated derivatives of vitamin B_6. Pyridoxamine phosphate, a form of vitamin B_6 known to occur naturally but previously found to be inactive as a coenzyme for transaminase, was shown by these studies to participate in transamination with α-keto acids to yield pyridoxal phosphate and the corresponding amino acids. Extension of these findings to other transamination systems led to the observation that the catalytic activity of pyridoxamine phosphate was equivalent to that of pyridoxal phosphate. The evidence is compatible with the concept that interconversion between enzyme-pyridoxamine phosphate and enzyme-pyridoxal phosphate complexes represents the basic mechanism of transamination.

Role of Glutamine and Asparagine in Transamination

Asparagine was probably the first amino acid to be obtained from a natural source (*10*), while glutamine was first isolated 77 years later in 1883 (*11*). Both amino acid amides occur free in plant and animal tissues and also exist in peptide linkage (for example, proteins, insulin, oxytocin). The presence of considerable concentrations of these amides in the free state in animal and plant tissues suggests that they play a significant role in cellular metabolism.

The present studies on the function of glutamine and asparagine in transamination represent a logical development of earlier observations on the metabolism of glutamine and glutamic acid. In 1935, Krebs reported that preparations of various animal tissues catalyzed the deamidation of glutamine to glutamic acid and, under certain conditions, the synthesis of glutamine from glutamic acid (*12*). Study of the latter reaction has shown that adenosine triphosphate supplies the energy for glutamine synthesis (*13, 14*), and more recently the mechanism of this synthesis has been the subject of further investigation (*15*).

The deamidation of glutamine was studied in detail by Greenstein and collaborators (*16–19*), who discovered two hepatic enzymes which catalyzed this reaction. One of these required phosphate or certain other anions for activity, whereas the other enzyme deamidated glutamine only in the presence of an α-keto acid. Analogous reactions involving asparagine were also observed.

In 1950, we undertook a study of the α-keto acid-dependent enzymatic deamidation of glutamine, using a purified liver enzyme that had been isolated by Errera (*19*). The major results of this investigation may be summarized as follows: (i) When L-glutamine and an α-keto acid were incubated with the enzyme, there was stoichiometric formation of ammonia, amino acid, and α-ketoglutaric acid. (ii) With glutamine that was labeled in the amide group with isotopic nitrogen, all the isotope was recovered as ammonia, unequivocally establishing the amide group as the source of ammonia (*20*). Thus,

$$\begin{array}{l} \mathrm{CON^{15}H_2} \\ | \\ (\mathrm{CH_2})_2 \\ | \\ \mathrm{CHNH_2} \\ | \\ \mathrm{COOH} \end{array} + \mathrm{RCOCOOH} \rightarrow \mathrm{RCHNH_2COOH} + \begin{array}{l} \mathrm{COOH} \\ | \\ (\mathrm{CH_2})_2 \\ | \\ \mathrm{C{=}O} \\ | \\ \mathrm{COOH} \end{array} + \mathrm{N^{15}H_3} \quad (1)$$

(iii) No ammonia was formed in the absence of an

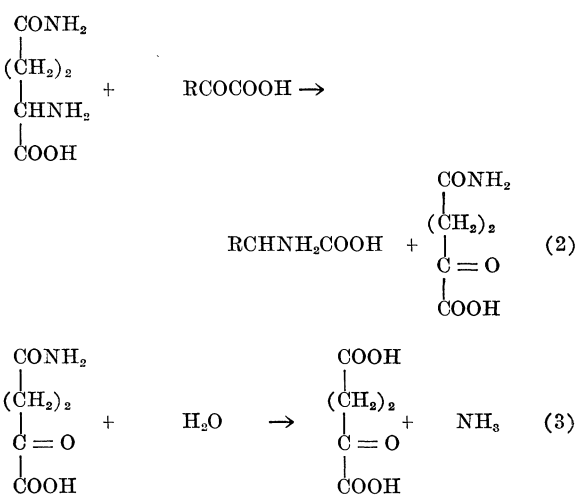
α-keto acid. (iv) With most of the α-keto acids studied, substitution of glutamine by glutamate resulted in a marked decrease or loss of transamination. (v) D-Glutamine was not active in this system.

These observations indicated that the deamidation and transamination phenomena were closely associated, and that deamidation did not precede transamination. It appeared possible that both reactions occurred simultaneously, or that transamination preceded deamidation. If the latter hypothesis were correct, it would be expected that α-ketoglutaramate would be formed as an intermediate, and that this α-keto acid-ω-amide would be either spontaneously or enzymatically deamidated to α-ketoglutarate.

In order to test this hypothesis, α-ketoglutaramate was prepared and studied in the enzyme system (*21*). Although α-ketoglutaramate was initially found to be only slowly deamidated by the enzyme preparation, it became apparent that this α-keto acid-ω-amide may exist in two interconvertible forms, only one of which exhibits properties characteristic of an α-keto acid. The α-keto analogs of glutamine and asparagine were prepared by enzymatic oxidation of L-glutamine and L-asparagine, respectively, by rattlesnake L-amino acid oxidase. In each case, the product obtained did not possess a reactive α-keto group as judged by ability to form a hydrazone and susceptibility to decarboxylation by hydrogen peroxide. Conversion of these products to forms that reacted readily with 2,4-dinitrophenylhydrazine and were decarboxylated by hydrogen peroxide occurred rapidly in alkaline solution. The reactive forms of α-ketosuccinamic and α-ketoglutaramic acids were enzymatically hydrolyzed by preparations of the glutamine transaminase and by a number of other tissue preparations. It was also of interest that the reactive form of α-ketosuccinamic acid, like many other α-keto acids (*22*), was reduced by lactic dehydrogenase in the presence of reduced diphosphopyridine nucleotide. Preparation and study of several related compounds (for example, α-ketoadipamic, α-keto-N-methylglutaramic, and α-keto-N-dimethylglutaramic acids) suggest that the ability to form such nonreactive structures depends upon a chain length of 4 or 5 carbon atoms and the presence of an ω-amide group in which at least 1 hydrogen atom is unsubstituted. The findings suggest a cyclic formulation for the structure of these compounds, proof of which must await further study.

The finding that α-ketoglutaramate was hydrolyzed by the enzyme preparation was compatible with the hypothesis that this keto acid was an intermediate in this reaction. However, attempts to isolate α-ketoglutaramate were not successful, because the activity of the amidase system was considerably greater than that of the transaminase, and also because of the relative instability of the latter system in the course of enzyme fractionation.

Another approach to the problem was therefore made in which a number of structural analogs of glutamine were studied in the hope of finding a substrate that would participate in transamination but whose α-keto analog would not be susceptible to attack by the amidase (*23*). These studies revealed the relatively strict specificity of the system for glutamine. For example, increasing or decreasing the length of the carbon chain (as in homoglutamine or asparagine) resulted in loss of activity. Substitution of the amide group in all but one instance yielded similar results. Of 22 compounds structurally related to glutamine, only three were active. One of these, γ-glutamylmethylamide, reacted with α-keto acids to yield the corresponding amino acid, α-ketoglutarate, and methylamine. On the other hand, two γ-substituted derivatives of glutamine, γ-methylglutamine and γ-methyleneglutamine (*24*), appeared to fulfill the desired requirements in that, although these amides transaminated with α-keto acids, no ammonia was formed in the course of the reaction. In the γ-methylglutamine-α-keto acid reaction, the formation of α-keto-γ-methylglutaramate was demonstrated, and a preparation of this keto acid, obtained by an independent procedure, was found to be insusceptible to the amidase activity. These results therefore suggest the following mechanism for the glutamine-α-keto acid reaction.



Although the γ-methylglutamine-α-keto acid reaction reached equilibrium at about 70 percent of maximal transamination, the glutamine-α-keto acid reaction proceeded to completion with stoichiometric formation of ammonia, a finding consistent with the expected tendency of the amidase system to force the reaction to completion by removal of α-ketoglutaramate.

The relatively narrow specificity for the amino group donor is in striking contrast to the large number of α-keto acids that are active as amino group acceptors (*20, 23, 25*). This is, therefore, a general transaminase system capable of catalyzing the formation of many of the natural amino acids. As is indicated in Table 1, almost all the keto acids studied were aminated. The failure of the keto analogs of isoleucine and valine to react suggests that the keto acid must have at least 2 β-hydrogen atoms in order to react,

and this conclusion is supported by the inactivity of trimethylpyruvate and α-ketophenylacetate. The α-keto analogs of lysine and ornithine may be insusceptible because of the tendency of these acids to cyclize to the corresponding piperidine and pyrroline derivatives (*26*).

Table 1. Amino acids formed by glutamine-α-keto acid transamination.

Alanine	Methionine
Glycine	Ethionine
Serine	Glutamic acid
α-Aminobutyric acid	Tryptophan
Norvaline	Asparagine
Norleucine	Cystine
Leucine	Arginine
Phenylalanine	Nitroarginine
Tyrosine	Cysteic acid
Cyclohexylalanine	Homoglutamine
α-Aminocaprylic acid	α-Aminoheptylic acid
ε-Hydroxy-α-aminocaproic acid	S-benzylcysteine
γ-Glutamyldimethylamide	ε-Carbobenzoxylysine
γ-Methylglutamine	δ-Carbobenzoxyornithine

The α-keto analogs of the following amino acids were inactive: valine, isoleucine, *tert.* leucine, α-phenylglycine, lysine, and ornithine.

In studies with the keto analog of arginine, the products included urea, glutamic-γ-semialdehyde, and glutamate, findings that may be ascribed to the presence of arginase and ornithine transaminase activities in the enzyme preparation (*27*). The sequence of events appears to be:

Glutamine + α-keta-δ-guanidinovalerate → arginine + α-ketoglutarate + ammonia (4)

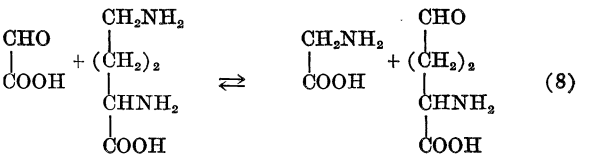
Arginine → ornithine + urea (5)

Ornithine + α-ketoglutarate → glutamic-γ-semialdehyde + glutamate (6)

SUM: Glutamine + α-keto-δ-guanidinovalerate → urea + ammonia + glutamate + glutamic-γ-semialdehyde (7)

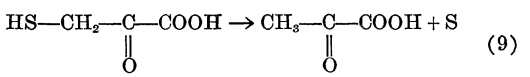
The reaction sequence could be stopped at reaction 5 by carrying out the experiments at *p*H 6, since reaction 6 does not occur at this value of *p*H. Furthermore, substitution of the keto analog of arginine with a nitro group in the amidine portion of the molecule resulted in the formation of nitroarginine, which accumulated and could be identified, since this derivative of arginine is not susceptible to attack by arginase.

The ornithine-α-ketoglutarate transamination reaction is atypical in that the δ-amino group reacts rather than the α-amino group. Nevertheless, ornithine also transaminated with other α-keto acids including pyruvic, α-ketobutyric, and glyoxylic acids, yielding glutamic-γ-semialdehyde, and the corresponding amino acids. The reaction between glyoxylate and ornithine is unique in that an aldehyde is both a reactant and a product:



(8)

An unusual finding that came to light in the course of studies on the α-keto acid specificity of the glutamine transaminase system was that alanine, rather than cysteine, was formed when β-mercaptopyruvic acid was incubated in the glutamine transaminase system. This result led to the finding of a new type of desulfuration reaction in which β-mercaptopyruvate was converted to pyruvate and sulfur (*28*):

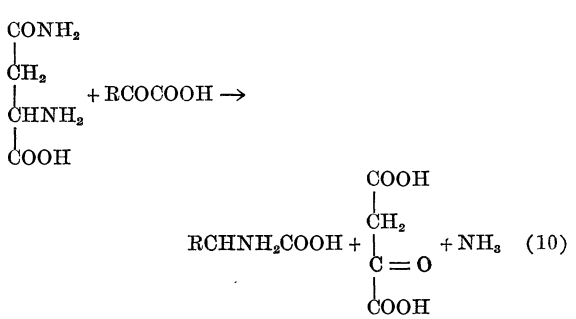


(9)

The enzyme that catalyzes this reaction is widely distributed and does not desulfurate cysteine or cystine. Transamination between β-mercaptopyruvate and glutamine to form cystine was demonstrated under conditions whereby appreciable desulfuration of the keto acid did not take place.

In contrast to cysteine desulfhydrase and glutamic-pyruvic transaminase activity, the glutamine-α-keto acid transaminase activity of rat liver was not significantly affected by vitamin-B_6 deficiency induced by dietary restriction of this vitamin and deoxypyridoxine administration (*29*). These findings do not necessarily exclude the participation of vitamin B_6 in the glutamine transaminase reaction, since the affinity of this enzyme for the coenzyme may be greater than that of the other systems studied. Further studies are necessary to elucidate this problem.

The existence of an asparagine-α-keto acid system, analogous to the glutamine-α-keto acid system, was suggested by the observation that the deamidation of asparagine was markedly increased in the presence of α-keto acids (*18*). Studies of this reaction revealed an associated transaminase reaction, whose specificity with respect to α-keto acids was similar to that of the glutamine system (*25*):



(10)

The asparagine-α-keto acid reaction appears to be catalyzed by a different enzyme than that responsible for the analogous reaction with glutamine, and in contrast to the latter system, the asparagine system was not found in kidney (*30*). Studies with the α-keto

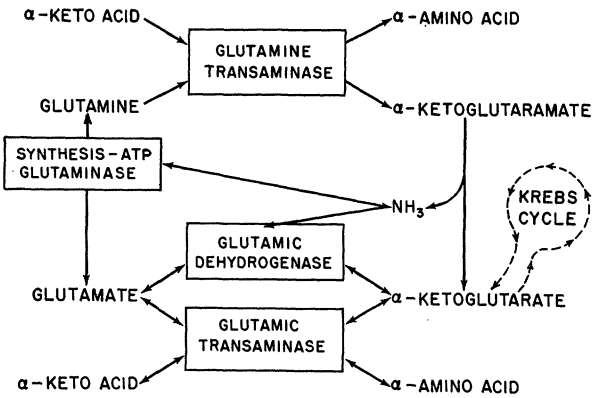


Fig. 1. Metabolic interrelationships involving glutamine.

analog of asparagine (α-ketosuccinamic acid) revealed that this compound was hydrolyzed to oxalacetate and ammonia by the enzyme preparation, a finding compatible with the participation of α-ketosuccinamic acid as an intermediate in the reaction (*21*). In the glutamine system, conversion of α-ketoglutaramate to glutamine could not be demonstrated, possibly because this amide was rapidly hydrolyzed to α-ketoglutarate and ammonia by this enzyme. Although α-ketosuccinamate was hydrolyzed by the asparagine transaminase preparation, it was possible, using high substrate concentrations, to exceed the capacity of the system to deamidate α-ketosuccinamate, and under these conditions, α-ketosuccinamate transaminated with a large number of L-amino acids to yield L-asparagine (*30*). D-Amino acids were not active in this system.

Some interrelationships between glutamine, asparagine, and their metabolites in liver are indicated in Figs. 1 and 2. The demonstration of the reversibility of α-ketosuccinamate formation raises the question of whether this keto acid may be a precursor in the biosynthesis of asparagine. The possibility that α-ketosuccinamate may be formed by a mechanism other than transamination remains to be investigated. Although glutamine is synthesized from glutamate, ammonium ion, and adenosine triphosphate, an analogous asparagine synthesis system has not yet been demonstrated.

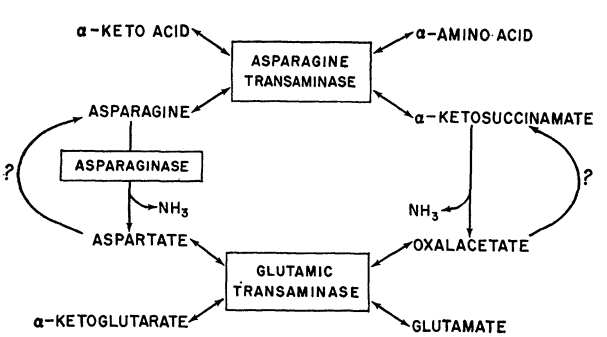


Fig. 2. Metabolic interrelationships involving asparagine.

Transamination Involving Isoleucine, Valine, Alanine, and α-Aminobutyric Acid

The demonstration of transamination reactions involving glutamine and asparagine, the participation of many α-keto acids in these reactions, and the finding in other laboratories of α-ketoglutarate-amino acid reactions (*31, 32*) suggested that the number of transaminases was considerably greater than was hitherto suspected. An interesting opportunity to examine the question of the multiplicity of transaminases arose as a result of the production of an unusual mutant strain of *Escherichia coli* by Bernard D. Davis. This organism required supplementation with L-isoleucine for growth, but in contrast to other isoleucineless mutants of *E. coli*, the growth of this mutant (No. 42–37) was not supported by the α-keto analog of L-isoleucine (*d*-α-keto-β-methylvalerate). A further point of interest was that supplementation with L-valine in addition to L-isoleucine was required for maximum growth, and here again the keto analog of valine (α-ketoisovalerate) did not serve in lieu of valine.

These growth phenomena suggested that the mutant organism lacked a transaminase capable of converting *d*-α-keto-β-methylvalerate to L-isoleucine, and this hypothesis was supported by the finding that cell suspensions and extracts of the mutant (in contrast to these of the wild strain of origin), were incapable of catalyzing reversible transamination between glutamate and α-keto-β-methylvalerate (*33*). However, although the enzymatic findings were compatible with the growth phenomena with respect to isoleucine, the results with valine were apparently contradictory. The enzyme studies showed that the mutant was incapable of reversible transamination between valine and α-ketoglutarate. However, although the growth studies revealed augmentation of growth in the presence of valine, considerable growth occurred in the absence of added valine. It therefore appeared that either valine was synthesized by a route not involving transamination, or that the organism possessed another enzyme capable of catalyzing transamination with valine. Examination of a large number of possible amino group donors revealed that only L-alanine and L-α-aminobutyrate were capable of reversible transamination with α-ketoisovalerate with extracts of the mutant strain, whereas this keto acid transaminated readily with these as well as with a number of other amino acids with preparations of the wild strain.

The evidence therefore indicated that at least three transaminase systems existed in the wild strain of *E. coli*: (i) an enzyme catalyzing transamination between glutaminate, isoleucine, valine, and several other aliphatic amino acids; (ii) a system catalyzing reactions between glutamate, aspartate, tryptophan, tyrosine, and phenylalanine; and (iii) a valine-alanine (or α-aminobutyric acid) system. The failure of the mutant to exhibit an absolute requirement for valine may be ascribed to the presence of the valine-alanine system. Confirmation of these results was obtained by physical separation of the three enzymes from extracts of the wild strain and by the finding that the valine re-

qurement of the mutant could be replaced by supplementation with equivalent amounts of either L-alanine or L-α-aminobutyric acid. Transamination pathways for the biosynthesis of isoleucine and valine in *E. coli*, consistent with the gene-enzyme concept, may therefore be formulated (Fig. 3).

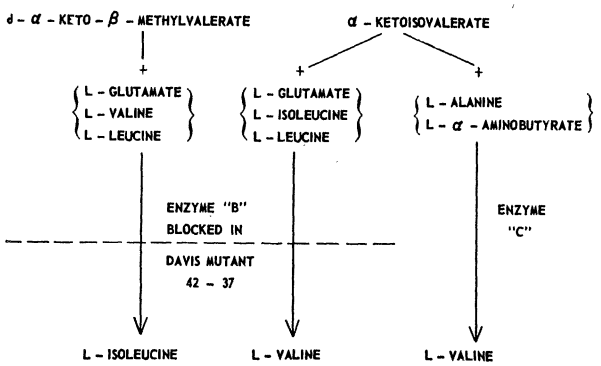


Fig. 3. Biosynthesis of isoleucine and valine by transamination.

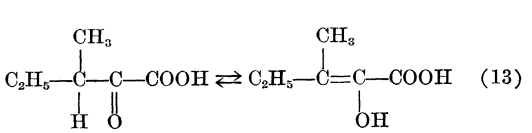
In the studies on the isoleucineless *E. coli* mutant (42–37) it became apparent that, of the four stereoisomers of isoleucine, only L-isoleucine was effective in promoting growth (Table 2). With other isoleucineless mutants of *E. coli* and with *Streptococcus faecalis*, L-isoleucine or *d*-α-keto-β-methylvalerate were capable of supporting growth, while the other three isomers of isoleucine and *l*-α-keto-β-methylvalerate were inactive. The strict stereospecific requirements of these organisms were in marked contrast to the behavior of *Lactobacillus arabinosus*, which responded to L-isoleucine, L-alloisoleucine, and both *d*- and *l*-α-keto-β-methylvalerate.

A study of enzymatic transamination with L-alloisoleucine and L-isoleucine, and the corresponding α-keto acid *isomers*, with preparations of *L. arabinosus* and of a hog heart preparation, revealed that the configuration of the β-carbon atom was not altered during the transamination reactions (*34, 35*):

L-Isoleucine + α-ketoglutarate →
L-glutamate + *d*-α-keto-β-methylvalerate (11)

L-Alloisoleucine + α-ketoglutarate →
L-glutamate + *l*-α-keto-β-methylvalerate (12)

On the other hand, it is clear that *L. arabinosus* possesses a mechanism for inversion of the β-carbon atom of L-alloisoleucine, since hydrolysates of organisms grown on L-alloisoleucine contain only L-isoleucine. Where the center of optical asymmetry resides at a carbon atom adjacent to a carbonyl group, enolization must result in racemization:



(13)

The rate of racemization of optically active α-keto-β-methylvaleric acid was found to increase rapidly with increasing alkalinity (*36*). The nature of the biological reaction is not known; formation of a phosphoenol derivative of the keto acid may be involved.

Table 2. Growth of several organisms on the stereoisomers and keto analogs of isoleucine.

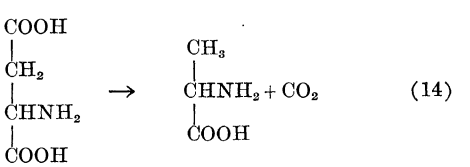
	E. coli (42–37)	*E. coli* (97–21)	*S. faecalis*	*L. arabinosus*	Rat
L-Isoleucine	+	+	+	+	+
L-Alloisoleucine	0	0	0	+	0
d-α-Keto-β-methylvalerate	0	+	+	+	+
l-α-Keto-β-methylvalerate	0	0	0	+	+
D-Isoleucine	0	0	0	0	0
D-Alloisoleucine	0	0	0	0	0

+ = growth ; 0 = no growth.

An inversion mechanism for *l*-α-keto-β-methylvalerate probably also exists in other species. Both isomers of α-keto-β-methylvalerate were found to support growth of weanling rats on an isoleucine-deficient ration, although the *l*-isomer was less effective than its *d*-enantiomorph (*37*). It is known that, of the four isomers of isoleucine, only L-isoleucine supports the growth of rats (*38*).

Relationship of Phosphorylated Derivatives of Vitamin B_6 to Transamination

The role of vitamin B_6 in enzymatic transamination was first suggested by Snell (*39*), and subsequent investigations have supported the concept that a phosphorylated derivative of vitamin B_6 functions as a coenzyme in transamination. Our initial studies involving pyridoxamine phosphate were carried out using a decarboxylase system of *Clostridium welchii*, which catalyzed the β-decarboxylation of L-aspartate to L-alanine (*40*).



(14)

This decarboxylase reaction proved to be unique in several respects. First, in contrast to the other amino acid decarboxylase reactions which have been described, the α-carboxyl was not attacked; second, the product was an α-amino acid; and finally, the reaction was activated by a large number of α-keto acids (as well as by pyridoxal phosphate). The activating effect of α-keto acids, although similar in some degree to the effect of α-keto acids on the deamidation of glutamine and asparagine, differed in one significant respect—that is, that catalytic, rather than stoichiometric, amounts of α-keto acids were required. Trans-

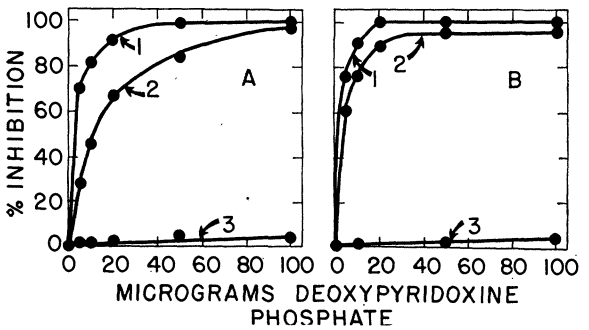


Fig. 4. Competition between coenzyme and deoxypyridoxine phosphate for the transaminase. *Curves 1*: Deoxypyridoxine phosphate was incubated with enzyme prior to addition of coenzyme. *Curves 2*: Deoxypyridoxine phosphate and coenzyme were added simultaneously. *Curves 3*: Enzyme was incubated with coenzyme prior to addition of deoxypyridoxine phosphate. A-pyridoxal phosphate; B-pyridoxamine phosphate. Experimental details are given elsewhere (*40*).

amination between the added α-keto acid and aspartate could be excluded from consideration, since alanine was the only amino acid formed, and because experiments with pyruvate labeled with radioactive carbon showed no incorporation of isotope into the alanine. A study of the activation of this decarboxylase system revealed that, after ultraviolet irradiation of the enzyme preparation, pyridoxal phosphate, but not α-keto acids, produced activation. Furthermore, such preparations were activated by pyridoxamine phosphate, provided that an α-keto acid was also present. These results suggested that the activating effect of α-keto acids on the decarboxylase system might be due to formation of pyridoxal phosphate by transamination between pyridoxamine phosphate present in the enzyme preparation and the added α-keto acid. Although further studies on purification of this enzyme are necessary in order to unequivocally prove this attractive hypothesis, these studies suggest the existence of an inactive enzyme-pyridoxamine phosphate complex, which may be converted by transamination with α-keto acids to an active enzyme-pyridoxal phosphate complex.

Although pyridoxal phosphate appears to represent an active coenzyme form of vitamin B_6 for decarboxylase activity and also for a number of other enzymatic reactions involving amino acids, it now appears that both pyridoxal and pyridoxamine phosphates are coenzymes for transaminase. Earlier studies in which pyridoxamine phosphate was found to be inactive may now be explained by the finding that combination of the enzyme with pyridoxamine phosphate takes place at a slower rate than enzyme-pyridoxal phosphate combination (*41*).

Preparation of crystalline pyridoxal phosphate, pyridoxamine phosphate, pyridoxine phosphate, and deoxypyridoxine phosphate (*42*), by Peterson and Sober (*43*) in this laboratory, made possible investigations on the coenzyme requirements of the purified pig heart glutamic-aspartic apotransaminase and on the mechanism of action of the inhibitor, deoxypyridoxine phosphate. As is indicated by the experiment described in Fig. 4, when the enzyme was incubated with either pyridoxamine phosphate or pyridoxal phosphate prior to addition of deoxypyridoxine phosphate, there was no inhibition. On the other hand, when incubation of the enzyme with deoxypyridoxine phosphate preceded addition of coenzyme, there was considerable inhibition. Similar results were observed with pyridoxine phosphate. The action of deoxypyridoxine phosphate (as well as pyridoxine phosphate), may therefore be ascribed to competition with the coenzyme for the enzyme. This type of inhibition, similar to that observed with the tyrosine decarboxylase system of *S. faecalis* (*44*), may explain, at least in part, the observed antagonism of vitamin B_6 by deoxypyridoxine in animals and microorganisms. Although deoxypyridoxine phosphate and pyridoxine phosphate can apparently combine with the enzyme, perhaps in part by linkages involving the phosphate group, these derivatives cannot participate in transamination.

The experimental findings with the phosphorylated vitamin B_6 derivatives are compatible with the following formulation, similar in principle to the original hypothesis of Snell, in which both pyridoxal phosphate and pyridoxamine phosphate function as coenzymes (*45*).

Pyridoxal phosphate (or pyridoxamine phosphate) + enzyme → enzyme-pyridoxal phosphate (or enzyme-pyridoxamine phosphate) (15)

Enzyme-pyridoxamine phosphate + α-ketoglutarate (or oxalacetate) ⇄ enzyme-pyridoxal phosphate + glutamate (or aspartate) (16)

It may be assumed that the rapid development of new knowledge of amino group transfer reactions during the last 3 or 4 years will continue for some time. Many of the tools and methods necessary for such further research are now available. It is clear from the wide scope of the transamination reaction that studies involving many amino donors and acceptors, as well as separation and purification of the catalytic systems involved, are minimal requirements for complete understanding of these metabolic phenomena.

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Analysis of Uranium in Sea Water

D. C. Stewart and W. C. Bentley

Argonne National Laboratory, Lemont, Illinois

To date, all the reported analyses of uranium in sea water have been based on the methods of Hernegger and Karlik (*1*). Uranium concentration (required because it is present in sea water only to the extent of a few parts in 10^9) has been accomplished by an elaborate series of coprecipitations with ferric hydroxide, and the final estimation has been carried out by use of the fluorophotometer. Sample sizes ranging from 200 ml to 22 lit in volume have been required.

In the present work (*2*), concentration has been accomplished by extracting the uranium directly from the sea water into an organic solvent containing dibutyl-orthophosphoric acid (DBP) as prepared by the method of Stewart and Crandall (*3*). Uranium estimation has been by fission-fragment counting of the U^{235} present by placing the sample in the Argonne heavy-water reactor. Routine sample sizes of volume 20 ml can readily be used, and, with special precautions, it should be possible to analyze accurately volumes of 1 ml or less.

The uranium is extracted by mixing the sample with 0.5 ml of 0.7–0.8*M* dibutyl-phosphoric acid in CCl_4. This is transferred *in toto* to a platinum counting plate, as is a subsequent "wash" of the same volume of CCl_4. After bringing the material to dryness and heating the plate in a flame, the sample is ready for counting. Standard plates carrying known amounts of uranium are counted at the same time, as are blank plates prepared directly from the reagents.

A preliminary test of the method was made, utilizing artificial sea water having the composition listed by Sverdrup, Johnson, and Fleming (*4*), ignoring components present to less than 0.01 percent; U^{233} tracer was added to produce 616 alpha counts per minute, per 50-ml sample. Six such samples were treated in this way to concentrate the uranium, but, in this case, the plates were alpha-ray counted rather than being put in the fission-fragment counter. The average recovery for the six samples was 94.5 percent ($s = 1.8$ percent).

Table 1. Recovery of added uranium from acidified natural sea water.

Sample	U (μg/lit)	
	Expected	Found
Unfortified 1953 Pacific Ocean water		2.36
		2.44
		2.43
		Mean = 2.41
Same + 0.33	2.74	2.82
	2.74	2.79
Same + 0.98	3.39	3.59
	3.39	3.53
Same + 1.77	4.18	4.18
	4.18	4.16
Same + 2.28	4.69	4.51
	4.69	4.55
Same + 3.26	5.67	5.60
	5.67	5.05