STRUCTURAL EVIDENCES FOR CHELATION AND SCHIFF'S BASE FORMATION IN AMINO ACID TRANSFER INTO CELLS*

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The present communication considers some findings which appear to implicate chelation in the amino acid concentration process. The inquiry grows out of the finding that pyridoxal stimulates concentration of amino acids by Ehrlich ascites tumor cells, and that cells from vitamin B₆-deficient mice show a deficient concentrating ability (4, 5). Metal chelation is known to be involved in the catalytic functioning of pyridoxal in non-enzymatic systems (6, 7) and at least in some enzymatic systems (8). Metal-amino acid-pyridoxal complexes similar to those shown in Fig. 1 are believed to be involved.

EXPERIMENTAL

Cells from mice inoculated 6 to 8 days previously were collected by centrifugation immediately before study and shaken at 37.5° in about six portions of Krebs-Ringer-bicarbonate medium containing the amino acid and any other agent under observation. These replaced an equivalent amount of sodium chloride. An atmosphere of 95 per cent oxygen and 5 per cent CO₂ was maintained. After 2 hours the cells were centrifuged and weighed and then extracted with picric acid when glycine was under study or with very dilute acetic acid at 100° (“hot water extract” (9)) for some of the other amino acids. The suspending fluid was freed of protein by the same means. Glycine, sarcosine, N-methylalanine, and γ-dimethylaminobutyric acid were determined by measuring the formaldehyde released by the action of ninhydrin (10). Methylamine and methylamino acids yield formaldehyde in this method; the yield, although less than 1 mole per N-methyl group, was sufficiently reproducible for present purposes.

The α-dimethylamino acids and N-methylproline were determined by

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taking advantage of their low reactivity to chloramine-T and their ability to form copper chelates as follows: 0.5 ml. of sample plus 0.5 ml. of 0.05 N chloramine-T plus 2 ml. of Woiwod's disodium phosphate solution (11) was held at 100° for 10 minutes, cooled, and then treated with Woiwod's copper phosphate suspension. The dissolved copper was then measured according to Woiwod's method. A 5 minute heating period was also satisfactory. The proportionality between the quantities of the N-dimethyl-amino acid added to tumor cell extracts and the resulting optical density is illustrated in Fig. 2 for N-dimethylglycine.

Fig. 1. Formation of chelate visualized in the order (1) metal coordination and (2) Schiff's base formation. Secondary amino acids presumably form the carbinolamine structure. Degradation of the amino acid is considered to proceed from tautomers, as at the right.

Fig. 2. Illustration of the proportionality between the quantity of N-dimethylglycine added to a cell extract and the optical density obtained by application of the method of Woiwod after destruction of other amino acids by chloramine-T.

Derivatives of $\alpha,\gamma$-diaminobutyric acid were measured by applying the colorimetric ninhydrin method (12) to the precipitate produced by phosphotungstic acid (13). The $\alpha$-amino-$\alpha$-methyl acids were determined on the basis of the increase in optical density upon increasing the time of heating with ninhydrin (12) from 10 to 60 minutes. This is made possible by their characteristically slow reaction with ninhydrin (9).

Study of Chelation—Determinations of absorption were made with the Beckman ultraviolet spectrophotometer and of pH with the Beckman model G pH meter. Electrometric titrations were made under nitrogen with 0.25 N sodium hydroxide for solutions 25 mm in amino acid and pyridoxal and 12.5 mm in nickel nitrate. The solutions were homogeneous over the pH ranges reported except that, with the combinations dimethyl-
glycine-nickel nitrate and dimethylglycine-pyridoxal-nickel nitrate, some precipitation occurred above pH 9.3.

L-\alpha,\gamma-Bis(methylamino)butyric Acid—L-\alpha,\gamma-Diaminobutyric acid was converted to the di-p-toluenesulfonyl (tosyl) derivative (m.p. 152–153°, from alcohol) as usual (14). The equivalent weight was confirmed by titration with alkali. 1 mmole of the product was treated in 2.5 ml. of 2 n sodium hydroxide with 4 mmoles of methyl iodide in a sealed tube at 70° (15). The product precipitated from ethyl acetate by petroleum ether was hydrolyzed in 1 ml. of 12 N HCl at 110° overnight. After thorough chilling the solution was removed from the crystals of p-toluenesulfonic acid, taken to dryness, and then titrated in aqueous solution to pH 6 with NaOH-activated Amberlite IR-4B resin. The aqueous solution of the amino acid monohydrochloride was concentrated in vacuo, and crystallization was induced by alcohol addition. The product, recrystallized from absolute alcohol with an over-all yield of 60 per cent, melted at 199–200° (all melting points corrected). With ninhydrin (16) the carbon dioxide yield was 99 per cent of theoretical.

r-p-Toluenesulfonamido-L-\alpha-amino-butyric Acid—5 ml. of a 0.6 mM solution of the copper salt of L-\alpha,\gamma-diaminobutyric acid (formed by boiling the monohydrochloride with copper carbonate) were treated with 1.5 ml. of 2 N NaOH and 2 ml. of ether. Six portions each of 1.5 mmoles of tosyl chloride and six portions each of 2.5 m.eq. of NaOH were added during shaking for about 15 hours (in a stoppered tube). The insoluble copper salt which formed was dissolved in dilute hydrochloric acid and treated with hydrogen sulfide, the copper sulfide was removed, and the solution was taken to dryness. Titration in aqueous solution with NaOH to pH 6 resulted in crystallization of the product, which was recrystallized from hot water in 38 per cent yield. The melting point was 200–201°, with decomposition; the carbon dioxide released by ninhydrin (15) was 99.8 per cent of theoretical.

r-p-Toluenesulfonamido-L-\alpha-aminobutyric Acid—The above product was benzoylated in NaOH as usual, and the product was recrystallized from alcohol by water addition. It melted at 151° and showed a neutralization equivalent of 373 as against the theoretical value of 376; yield 90 per cent of theoretical.

\gamma-Methylamino-\alpha-aminobutyric Acid Monohydrochloride—1 mmole of the preceding product was dissolved in 1.55 ml. of 2 N NaOH and treated with 2 mmoles of methyl iodide as above. Hydrolytic removal of the tosyl and benzoyl groups and conversion to the monohydrochloride were performed as recorded above, and recrystallization was from 95 per cent ethanol; m.p. 201–203°. A degree of racemization during methylation is likely, although optical purity was not examined. Since this product was strongly
concentrated by cells in spite of the possible presence of the D form, a preparation by a route excluding racemization was not considered necessary.

γ-Amino-L-α-dimethylaminobutyric Acid Monohydrochloride—Because of its low solubility γ-p-toluenesulfonamido-α-aminobutyric acid was reductively methylated at a dilution of 1 mmole per 10 ml. and at 3 atmospheres of hydrogen. The ninhydrin color reaction was essentially abolished by this procedure. Removal of the tosyl group and crystallization of the amino acid monohydrochloride were performed as above with 80 per cent recovery. The melting point (from 95 per cent alcohol) was 213–215° with decomposition. Reaction with ninhydrin failed to evolve CO₂.

Reductive Methylation—N-Dimethylglycine, N-methyl-L-proline, and γ-dimethylaminobutyric acid were prepared by the action of formaldehyde and formic acid (17). N-Methylproline hydrochloride was crystallized from glacial acetic acid and melted at 188° with decomposition. γ-Dimethylaminobutyric acid hydrochloride was crystallized from alcohol in 48 per cent yield and recrystallized from acetone to give large crystals melting at 151–152°. DL-α-Aminobutyric acid, L-α,γ-diaminobutyric acid, and γ-p-toluenesulfonamido-α-aminobutyric acid were methylated with hydrogen and formaldehyde with a charcoal-palladium catalyst according to Ingram (18) by the method of Bowman and Stroud (19). In each case chromogenicity to ninhydrin (12) was essentially eliminated. DL-α-Dimethylaminobutyric acid, crystallized from a few drops of anhydrous ethanol by ethyl acetate addition, melted at 173–175°; Friedmann records 179° (20). The bis(dimethylamino)butyric acid monohydrochloride, needles obtained from absolute alcohol, decomposed upon heating to 203°.

The N-methyl derivatives of α-aminoisobutyric acid were readily obtained by the Strecker synthesis (21) from acetone and methylamine or dimethylamine. α-N-Dimethylaminoisobutyric acid hydrochloride melted at 246–247°.

RESULTS AND DISCUSSION

Effect of Spacing between Amino and Carboxyl Groups—Both α- and β-amino acids are concentrated. β-Alanine (22) is concentrated essentially as well as α-alanine (23). But, as will be shown below, γ-aminobutyric acid is not concentrated to a significant extent. Here is a very close parallelism to the stability of the copper salts of the amino acids, a situation which might be explained by chelation during transfer.

Primary, Secondary, and Tertiary Amino Acids—From the preceding result one might suggest that a heavy metal is the amino acid carrier. There is, for example, the known association between amino acid distribution and copper metabolism in Wilson’s disease. This simple theory appears
to be excluded by the finding that $N$-dimethylglycine is only slightly concentrated, and $\alpha$-dimethylaminoisobutyric acid, $N$-methyl-$L$-proline, and $N$-dimethylamino-$n$-butyric acid are not concentrated at all (Fig. 3). Sarcosine, $N$-methylalanine, proline, hydroxyproline, and $\alpha$-methylaminoisobutyric acid are concentrated to substantial extents. These amino acids form chelates readily, for example copper salts. Apparently the amino group not only must be near enough to the carboxyl group, but also

![Graph showing concentration ratios of various amino acids](http://www.jbc.org/)

**Fig. 3.** Comparison of concentrative transfer of primary, secondary, and tertiary amino acids by ascites cells. Time 2 hours; initial amino acid levels 20 to 30 mM for the suspension and uniform for each curve. The curve marked glycine illustrates the comparison of the concentration of glycine, sarcosine, and dimethylglycine. The curve marked $\alpha$-aminoisobutyric shows the effect of $N$-methylation of this amino acid. The lowest curve compares the concentration of $L$-proline and $N$-methyl-$L$-proline.

must be either primary or secondary. It was recognized earlier (24) that an acylamino group would not serve.

As soon as the failure of tertiary amino acids to be concentrated was appreciated, it was suspected that they would be innocuous to the transfer of other amino acids. In general strongly concentrated amino acids are strong antagonists to the transfer of others (9). Table I illustrates verification of this idea; adding a second $N$-methyl group to $\alpha$-aminoisobutyric acid terminated its inhibitory action on glycine concentration. This finding probably eliminates a possible explanation entertained earlier for the inhibitory action of ethylenediaminetetraacetic acid; namely, that being itself an amino acid it competes with other amino acids for the carrier. As a tertiary amino acid it probably cannot bind the carrier, and more likely the competition is for a metal ion.
The linkage anticipated between pyridoxal and an amino acid, namely Schiff's base formation, can occur with either primary or secondary amino groups. In the former case the aldimine and carbinolamine forms are both possible, whereas the Schiff base of the secondary amino group is restricted to the carbinolamine form (Fig. 1). The present results, except perhaps for the apparent small concentration of dimethylglycine, are compatible with the formation of metal-stabilized Schiff’s bases in the transfer of amino acids.

Two methods, absorption and titrimetric, were used to investigate complex formation between amino acid, pyridoxal, and metal ion. Fig. 4

**Table I**

Comparison of N-Methyl- and N-Dimethyl-α-aminoisobutyric Acids As Inhibitors of Amino Acid Concentration

In Experiment 322 the distribution of the endogenously present amino acids was observed after 2 hours by the colorimetric ninhydrin method. When the inhibitor was absent, the final cellular concentration found was 73 mM for the cell water and 5.0 mM for the suspending fluid. In Experiment 323 glycine was added to produce a concentration of 1.8 mM in the suspension; the time of incubation was 1 hour.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Test amino acid</th>
<th>Initial inhibitor concentration (mM)</th>
<th>Distribution ratio, test amino acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Distribution ratio, test amino acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No inhibitor</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N-Methyl</td>
</tr>
<tr>
<td>322</td>
<td>All endogenous</td>
<td>32</td>
<td>14.6</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>63</td>
<td>14.6</td>
</tr>
<tr>
<td>323</td>
<td>Glycine</td>
<td>1.8</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>9.0</td>
<td>10.0</td>
</tr>
</tbody>
</table>

shows the increase in absorption at about 900 m\(\mu\), characteristic for the aldimine structure, which appears when pyridoxal and glycine-nickel salt are mixed in neutral solution (25). With sarcosine only small changes in absorption occur in this region, although chelation is made evident by the pH changes described below. Under the same circumstances N-dimethylglycine-nickel salt plus pyridoxal shows an absorption essentially unchanged from that calculated for the physical mixture.

Whereas complex formation between a neutral α-amino acid and a metal ion leads to displacement of a hydrogen ion (Reaction 1, Fig. 1), the combination with pyridoxal (Reaction 2), if subsequent, does not. Nevertheless, there are characteristic decreases in pH (and therefore shifts in the position of the titration curve) upon the addition of pyridoxal, attributable to increases in the stability of the amino acid-metal complex, bringing Re-
action I nearer to completion. If the reactions are visualized in the opposite order, the displacement of the hydrogen ion would be attributed to Schiff's base formation. In Fig. 5 the experimental titration curve for the mixture of glycine-nickel salt and pyridoxal is compared with the predicted titration curve, based on the separate titrations of glycine-nickel salt and of pyridoxal. By the same method, a similar interaction is established between sarcosine-nickel salt and pyridoxal, whereas no reaction occurs between nickel-dimethylglycine and pyridoxal, or at least no reaction involving a change in the stability of the metal-nitrogen bond.

![Absorption changes on mixing amino acid-nickel salts and pyridoxal.](http://www.jbc.org/)

The foregoing results indicate that for active biological transfer it is not enough for the amino group to lie within a specified distance from the carboxyl group; it must also be a primary or secondary amino group. Furthermore, these structural requirements correspond to the requirements for the formation of a metal-stabilized Schiff's base.

**Role of Aromatic Hydroxyl Group and Methylol Group of Pyridoxal—** Phosphorylation of the 5-methylol group of pyridoxal is probably not involved in the present stimulatory activity: first, because pyridoxal phosphate is, if anything, less active than pyridoxal (5), and, secondly, because the benzene analogue of pyridoxal, 4-nitrosalicylaldehyde, is also strongly stimulatory (Fig. 6). Furthermore, 5-deoxypyridoxal\(^1\) is quite as active in this respect (7).

\(^1\) Gift of Dr. Karl Pfister, 3rd, Merck and Company, Inc.
The 3-hydroxyl group, in contrast, is essential to the action of pyridoxal, as shown by the inactivity of the 3-methoxy analogue\(^1\) (Table II). This phenolic group is well known to be involved in the complex formation of the type under consideration (cf. \((26)\)). It exists in the dissociated (phenolate) form at ordinary pH values, a pK of 4.23 having been found \((27)\).

Salicylaldehyde is inactive in the transfer process; 4-deoxypyridoxine is

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**TABLE II**

**Illustration of Lack of Stimulatory Action by 3-O-Methylpyridoxal**

The aldehyde was added to the cell suspension at a 0.9 mM level and the amino acid at a 2 mM level. The recorded distribution ratios, the cell glycine level to extracellular level, were obtained after 1 hour.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Amino acid studied</th>
<th>Amino acid distribution found</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No addition</td>
<td>Pyridoxal added</td>
</tr>
<tr>
<td>326</td>
<td>Glycine</td>
<td>8.0</td>
</tr>
<tr>
<td>327</td>
<td>Sarcosine</td>
<td>11.2</td>
</tr>
</tbody>
</table>

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Fig. 5. Titration changes on mixing amino acid-nickel salts and pyridoxal. For each amino acid the curve on the right represents the sum of the titration of the amino acid-nickel salt and the titration of the pyridoxal. The curve at the left represents the actual titration curves obtained for the mixture. With dimethylglycine the titrations were indistinguishable.

Fig. 6. Effects of pyridoxal, 4-nitrosalicylaldehyde, and 5-deoxypyridoxal on glycine concentration by the ascites cells. The relative distribution ratio is 100 times the ratio of the cell glycine to the extracellular glycine level in the presence of an agent divided by the distribution ratio in the absence of the agent. \(\triangle\), pyridoxal; \(\times\), 5-deoxypyridoxal; \(\bigcirc\), 4-nitrosalicylaldehyde.
also inactive (5). At higher levels it becomes inhibitory, but this is also true of pyridoxal. The requirement for three characteristic structures for stimulatory action (the aldehyde group, the phenolic hydroxyl group, and the nitro group or ring nitrogen) is understandable in terms of Reaction 2 of Fig. 1.

Rôle of \( \alpha \)-Hydrogen—The labilization and migration of the \( \alpha \)-hydrogen of the amino acid are considered necessary for many degradative reactions catalyzed by pyridoxal derivatives (see the reactions in Metzler et al. (6)). \( \alpha \)-Aminoisobutyric acid, for example, which lacks an \( \alpha \)-hydrogen, appears to escape degradation when it is fed to animals. This amino acid, how-

### Table III

**Concentration by Carcinoma Cells of Amino Acids Lacking an \( \alpha \)-Hydrogen**

The analytical results represent essentially quantitative recoveries of the amino acid added. About 200 mg. of cells were taken per ml. of suspension. The time of incubation was 2 hours.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Cellular</th>
<th>Extracellular</th>
<th>Distribution ratio</th>
<th>Gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha )-Methyl-( DL )-methionine</td>
<td>61.1 ( \mu )M</td>
<td>18.5 ( \mu )M</td>
<td>3.30</td>
<td>43</td>
</tr>
<tr>
<td>( DL )-Ethionine</td>
<td>54.4 ( \mu )M</td>
<td>18.0 ( \mu )M</td>
<td>3.02</td>
<td>36</td>
</tr>
<tr>
<td>( DL )-Methionine</td>
<td>54.9 ( \mu )M</td>
<td>19.1 ( \mu )M</td>
<td>2.87</td>
<td>36</td>
</tr>
<tr>
<td>( \alpha )-Aminoisobutyric acid</td>
<td>52.2 ( \mu )M</td>
<td>17.0 ( \mu )M</td>
<td>3.07</td>
<td>35</td>
</tr>
<tr>
<td>( \alpha )-Methyl-( DL )-serine</td>
<td>73.9 ( \mu )M</td>
<td>18.1 ( \mu )M</td>
<td>4.08</td>
<td>56</td>
</tr>
<tr>
<td>( \alpha )-Methylolserine</td>
<td>56 ( \mu )M</td>
<td>18 ( \mu )M</td>
<td>3.1</td>
<td>38</td>
</tr>
<tr>
<td>( \alpha )-Methyl-( DL )-( \alpha )-aminobutyric acid</td>
<td>71.9 ( \mu )M</td>
<td>17.7 ( \mu )M</td>
<td>4.06</td>
<td>54</td>
</tr>
<tr>
<td>( \alpha )-Methyl-( DL )-aspartate</td>
<td>9.76 ( \mu )M</td>
<td>22.4 ( \mu )M</td>
<td>0.44</td>
<td>-13</td>
</tr>
<tr>
<td>( \alpha )-Methyl-( DL )-asparagine</td>
<td>53.6 ( \mu )M</td>
<td>13.6 ( \mu )M</td>
<td>3.94</td>
<td>40</td>
</tr>
<tr>
<td>( \alpha )-Methyl-( DL )-glutamate</td>
<td>7.61 ( \mu )M</td>
<td>20.6 ( \mu )M</td>
<td>0.37</td>
<td>-13</td>
</tr>
</tbody>
</table>

ever, is more strongly concentrated by the carcinoma cells than analogous straight chain amino acids. This is true also for \( \alpha \)-methyl-\( DL \)-methionine,\(^1\) \( \alpha \)-methyl-\( DL \)-serine,\(^1\) \( \alpha \)-methylolserine, \( \alpha \)-methyl-\( DL \)-\( \alpha \)-aminobutyric acid, and \( \alpha \)-methyl-\( DL \)-asparagine\(^1\) (Table III). In the rat \( \alpha \)-aminoisobutyric acid, \( \alpha \)-methylserine, and \( \alpha \)-methylolserine (bis(\( \alpha \)-methylol)glycine) are concentrated by various tissues and as a consequence lost slowly from the organism (28). In contrast to the six neutral amino acids investigated, the dicarboxylic acids, \( \alpha \)-methyl-\( DL \)-aspartic acid\(^1\) and \( \alpha \)-methyl-\( DL \)-glutamic acid,\(^1\) are not concentrated at all (Table III). On comparing \( \alpha \)-methyl-asparagine and \( \alpha \)-methylaspartic acid, it appears that the \( \alpha \)-hydrogen group becomes necessary for transfer when the \( \beta \)-carboxyl group is free. That the dicarboxylic and neutral amino acids belong to two different classes with regard to transfer has been recognized; competition occurs only
within the classes (9). Conceivably, transamination may even be involved in the apparent accumulation of glutamate by these cells.

If complexes similar to those of Fig. 1 are involved in the transport process for neutral amino acids, clearly they do not need to tautomerize to the imino acid form (lower right section, Fig. 1). In fact, one would conclude that such structural shifts are disadvantageous to concentrative transfer. The prevention of further rearrangement of the aldime form may explain the stronger transfer of amino acids lacking the α-hydrogen.

Role of Second Nitrogenous Group in Amino Acid Structure—Additional evidence for chelation is supplied by the behavior of α,γ-diaminobutyric acid and α,β-diaminopropionic acid. These, especially the former, are so strongly concentrated as to be destructive to the cell; the organic cation rapidly displaces the cell potassium and causes tremendous swelling (29). The abrupt increase in activity in the series of diamino acids in going from ornithine to diaminobutyrate (Fig. 7) seems to be best explained on steric grounds; α,γ-diaminobutyric acid and α,β-diaminopropionic acid are the only members of the series which can form stable rings of five or six members involving the two amino groups and a metal ion. Albert has shown titrimetrically that the copper salts of these two amino acids involve mainly both amino groups, and to a smaller extent the α-amino group with the carboxyl group, whereas the copper salts of ornithine and lysine have largely the usual structure (30).

In the same way the Schiff bases of these two diamino acids are probably chelated to both nitrogens as illustrated in Fig. 8. By using Job's method of continuous variation (31, 32) the highest absorption was obtained from pH 4.8 to 7.2 when the molar ratio nickel-pyridoxal-diamino acid was 1:2:2. At pH 10.4, however, the combination in the proportions 1:2:1 absorbed much more strongly (Fig. 9). An absorption maximum occurred at about 940 mυ in all cases, supporting an aldime structure. These results support the composition Ni(pyridoxal)₂(diamino acid)₂ at pH 7.2 and Ni(pyridoxal)₂ diamino acid at pH 10.4. The former probably resembles Structure A, Fig. 8, with uncertainty as to which nitrogen participates in Schiff's base formation. The γ-amino group is perhaps more likely, judging from the dissociation constants (30), from the experience with ornithine transamination (33, 34), from the studies by Witkop and Beiler on the infra-red spectra of the pyridoxylidene derivatives of lysine and ornithine (35), and from the preference shown for the γ-amino group in acylations (36).

The composition, Ni(pyridoxal)₂diamino acid, probably corresponds to the double Schiff base (Structure B, Fig. 8) analogous to the well studied metal-salicylaldehyde-ethylenediamine chelates (cf. (26)). Chelates of the above two compositions were readily prepared and separated from aqueous
methanol (37), thus confirming the conclusions from the absorption measurements.

Under biological conditions the monopyridoxylidene derivatives of the

diamino acids would be the more likely to occur, considering the pH and the relatively low pyridoxal levels.

Concentrative Transfer of Derivatives of Diaminobutyric Acid—The above results probably explain the failure of bis(methylamino)butyric acid to be well concentrated by the tumor cells (Table IV), because this amino
acid would be expected to form a quite different derivative with pyridoxal and metals, presumably an imidazolidine derivative. The characteristic aldimeine absorption at about 940 m\(\mu\) was not obtained with it in the presence of pyridoxal and nickel. A single methyl group on the \(\gamma\)-amino group abolished neither aldimeine formation nor biological transfer. Two \(N\)-methyl groups at the \(\alpha\) position, however, almost eliminated concentrative uptake (Table IV), although \(\gamma\)-amino-\(\alpha\)-dimethylaminobutyric acid still formed a chelate of aldimeine structure with pyridoxal and nickel.

Blocking of the \(\gamma\)-amino group to Schiff’s base formation is well illustrated by 2-pyridyl-\(\mathrm{DL}\)-alanine, which is very well concentrated (5) and chelates readily with pyridoxal and nickel. Despite the likelihood that the \(\gamma\)-amino group may react more readily with pyridoxal in the test-tube, it is concluded that abolition of the possibility of Schiff’s base formation with this amino group does not greatly diminish biological transfer, whereas abolition of the possibility of reaction by the \(\alpha\)-amino group is critical.

**SUMMARY**

1. The structural features which influence the biological transfer of amino acids into the Ehrlich ascites carcinoma cells (position of the amino group; primary, secondary, or tertiary state of the amino group; position of a second amino group; tertiary state of that amino group) also modify the reaction with pyridoxal in the presence of metals in a manner consistent with the participation of this reaction in the transfer process.

2. A structural feature which prevents tautomerization of the pyridoxylidene derivative of the amino acid intensifies its biological transfer.
Three structural features of aromatic o-hydroxyaldehydes which are necessary for the stimulation of amino acid transfer are pertinent to the formation and stability of chelated Schiff's bases with amino acids.

4. Tertiary $\alpha$-amino acids do not interfere with the concentration of other $\alpha$-amino acids. Accordingly, the inhibitory action of ethylenediaminetetraacetate must be upon another basis.

5. L-$\alpha$,y-Diaminobutyric acid forms both mono- and bispyridoxylidene chelates containing 1 nickel atom for each 2 pyridoxal residues and giving absorption evidence of the aldimeine structure. The relative stabilities of these chelates depend upon the pH.

6. Blockage by methylation of aldimeine formation at both amino groups or of Schiff's base formation at the $\alpha$-amino group largely abolishes concentrative transfer of diaminobutyric acid into cells. The presence of a $\gamma$-amino group, however, stimulates concentrative transfer, even though it may be tertiary.

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