Ammonia Nitrogen Produced From Isomeric Peptides in Kidney Homogenate Digests

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On aerobic incubation of glycyl-*dl*-alanine and of *dl*-alanylglycine with aqueous homogenates of rat kidney tissue, we noted that considerable ammonia accumulated in digests of the former peptide while little or none appeared in those of the latter. Essentially similar findings were observed with isomeric peptides of *dl*-leucine (Table 1). Of further interest was the fact

TABLE 1

AMMONIA N PRODUCED FROM PEPTIDES AND AMINO ACIDS IN AEROBIC DIGESTS OF RAT KIDNEY HOMOGENATES*

Substrate	Hours of incubation	Ammonia N in micromoles†
dl-Alanine	4	6
"	8	10
Glycyl-*dl*-alanine	4	6
"	8	10
dl-Alanylglycine‡	4	<1
"	8	1
dl-Leucine	4	5
"	8	9
Glycyl-*dl*-leucine	4	5
"	8	9
dl-Leucylglycine	4	<1
"	8	1
l-Leucine	4	1
Glycyl-*l*-leucine	4	1
Glycine	8	0
Glycylglycine	8	0
dl-Valine	4	5
dl-Isovaline	4	0
dl-Leucylglycylglycine	4	0
Glycyl-*dl*-leucylglycine	4	0
Glycylglycyl-*dl*-leucine	4	5
d-Leucylglycine§	4	0
d-Leucylglycine + 0.001 M $MnCl_2$	4	1
d-Leucylglycine + Mn + *l*-leucine	4	<1
d-Leucylglycylglycine	4	0
d-Leucylglycylglycine + 0.001 M $MnCl_2$	4	0

* Digests consisted of 1 cc. of dialyzed homogenate equivalent to 333 mg. of tissue, plus 2 cc. of 0.15 M borate buffer at pH 8.1, plus 1 cc. of 0.05 M racemic or 0.025 M optically active substrate. Enzymatic activity was measured by the amount of ammonia produced, corrected for the extract blanks. No ammonia was produced from any substrate when the digestion was conducted under anaerobic conditions. Temperature, 37° C.

† Theoretical maximum, 25 micromoles from each optically active component.

‡ Chloroacetyl-*dl*-alanine and chloroacetylglycyl-*dl*-leucine in similar aerobic digests yielded no ammonia N.

§ $[\alpha]_D = -82°$.

that the ammonia which appeared in digests of glycyl-*dl*-alanine and of glycyl-*dl*-leucine was close in order of magnitude to that which appeared in digests of *dl*-alanine and *dl*-leucine, respectively. Of the isomeric tripeptides studied, only glycylglycyl-*dl*-leucine yielded ammonia.

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The ammonia which appears from the racemic substrates under these experimental conditions is due principally to *d*-amino acid oxidase activity and may be related specifically to the oxidative desamination of the *d*-amino acid moiety of the peptides (*5*). Neither *l*-leucine nor glycyl-*l*-leucine yields appreciable ammonia under these conditions. That the oxidative desamination involves the α-β hydrogen atoms of the substrate is revealed in the relative susceptibility of *dl*-valine and of *dl*-isovaline (Table 1). The latter possesses a tertiary carbon atom. Manometric procedures of estimating peptidase activity through amino acid oxidase have been reported (*4, 7*).

The contribution of the *l*-amino acid components to the yield of ammonia in digests of the racemic, isomeric peptides may therefore be relatively neglected, and the role of the natural *l*-peptidase in the splitting of the *l*-form of the peptides in the kidney digests is not of immediate concern in the interpretation of the phenomena. This leaves for consideration only the *d*-form of the peptides, namely, *d*-alanylglycine and *d*-leucylglycine on the one hand, and glycyl-*d*-alanine and glycyl-*d*-leucine on the other. Two alternative explanations for the behavior of the isomeric peptides may be offered.

(1) *d*-Amino acid oxidase may be considered as acting only on free *d*-amino acids, and therefore the ammonia noted in digests of the racemic peptides could only have arisen subsequent to the action of *d*-peptidase on the peptides liberating the free amino acids. On this basis, it would appear that glycyl-*d*-alanine and glycyl-*d*-leucine were very susceptible, whereas *d*-alanylglycine and *d*-leucylglycine were relatively resistant, to the action of *d*-peptidase. This would not be in agreement with the relative susceptibility of the corresponding *l*-peptides to intestinal dipeptidase, whereby alanylglycine is hydrolyzed at twice the rate as glycylalanine (*6*). A slight, but definite, manganese-activatable *d*-leucylpeptidase activity is noted in the rat kidney digests (Table 1) (cf. *7*). This activity is slightly depressed by the addition of *l*-leucine.

(2) *d*-Amino acid oxidase may be considered as acting not only on the free *d*-amino acids, but also upon *d*-amino acids bound through the amino group in peptide linkage with another amino acid. Such a concept is in harmony with Bergmann's view that the oxidative desamination of amino acids might be effected while they are in the peptide chain, yielding by an α-β dehydrogenation the corresponding dehydropeptide, which subsequently is split by dehydropeptidases to products which include ammonia and keto acids (*1*). Thus:

$$(1)\quad RCH(NH_2)CONHCH(CH_2R^1)COOH \xrightarrow[\text{Oxidase}]{-2H} RCH(NH_2)CONHC(=CHR^1)COOH \xrightarrow[\text{Dehydropeptidase}]{H_2O} RCH(NH_2)COOH + NH_3 + CH_2R^1C(=O)COOH$$

$$(2)\quad 2H + O \longrightarrow H_2O.$$

On this basis, dipeptides which contain a glycine residue at the carboxyl end of the chain, as in alanylglycine and leucylglycine, could not form dehydropeptides, whereas peptides like glycylalanine and glycylleucine could form such α-β unsaturated peptides. Kidney tissue is, of all animal tissues, richest in both *d*-amino acid oxidase (*5*) and dehydropeptidase (*2*).

The second of these alternatives was sympathetically considered by Krebs in his early work on the subject of amino acid oxidation, but no decision was reached by him (*5*). On the basis of our present data, we are inclined to favor this second alternative, which is not only consistent with the Bergmann concept of intracellular peptide metabolism, but also supplements earlier work from this laboratory on the enzymatic susceptibility of peptides of *l*-cystine (*3*). In the final analysis, however, the Bergmann concept can only be proved by separation of the enzymes involved, and work on this possibility is in progress.

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Use of Insoluble Penicillin Salts for the Prolongation of Penicillin Blood Levels

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Many methods have been proposed for prolonging blood levels of penicillin. The one usually employed in medical practice is that proposed by Romansky and Rittman (*3*), namely, an intramuscular injection of a suspension of calcium penicillate in beeswax and a vegetable oil. This method has the disadvantage of using a substance (beeswax) of variable composition which may not in all cases be completely absorbable.

In a search for a method of prolonging penicillin blood levels, the writer decided to investigate the insoluble penicillin salts. These compounds have not been used until now because it was believed that the penicillin in such salts was irreversibly inactive. Thus, Abraham and Chain (*1*) found that penicillin was inactivated by a large number of metallic ions—copper, lead, zinc, cadmium, nickel, mercury, and uranium. They also reported that no activity could be recovered by decomposing the inactivated material with acid and extracting with ether. Bacharach and Hems (*2*) state that zinc, copper, mercury, and lead inactivate penicillin rapidly and iron less rapidly. Whether this inactivation is due to the formation of an insoluble penicillin or whether there is a definite chemical change in the penicillin is a subject for future investigation.

It occurred to the writer that there was a possibility that the inactivated insoluble penicillin might be reactivated *in vivo*. If this proved to be true, the insoluble salt would be more slowly absorbed than the soluble sodium, potassium, and calcium salts now in use and would therefore result in a marked prolongation of blood levels. Moreover, all the substances used would be completely absorbable. These suppositions were correct, as shown by the following data.

A control intramuscular injection in a rabbit of 20,000 units/kg. of penicillin suspended in peanut oil gave no readable blood level after 5 hours. On the other hand, a similar injection of silver penicillate produced a blood level of .08 units/cc. at 17 hours and .03 units at 20 hours; one of mercury penicillate, a level of .08 units at 17 hours and one of ferric penicillate, a level of .16 at 17 hours and .02 at 20 hours.

Penicillin produces insoluble salts with iron, copper, tin, vanadium, lead, lanthanum, cesium, zirconium, mercury, bismuth, silver, gold, and probably many other metals. Insoluble salts are also obtained with numerous organic substances, basic or cationic in character, such as the triphenylmethane dyes, namely, gentian violet, brilliant green, crystal violet, methyl violet, and basic fuchsin; with the acridine dyes such as acriflavine and proflavine; with Nile blue, malachite green, toluylene red, safranine, quinine, quinidine, cinchonine, cinchonidine, and hyamine 1622.

The reactivation of penicillin *in vivo* takes place not only with inorganic but also with organic salts. An intramuscular injection in a rabbit of 20,000 units/kg. of brilliant green penicillate produced a blood level of .16 units/cc. at 18 hours, and a similar injection of gentian violet penicillate, a blood level of .04 units at 18 hours.

A more detailed report will appear elsewhere.

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Inhibition of the Enzymatic Hydrolysis of ATP by Certain Cardiac Drugs[1]

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Although many investigations on the action of cardiac drugs have been carried out, only a few studies have dealt with their influence on the enzymatic reactions of heart muscle. Recently, however, Guerra, *et al.* (*2*) have reported that $1:10^6$ ouabain increased the liberation of inorganic phosphorus from adenosine triphosphate (ATP) as catalyzed by a cardiac muscle myosin preparation.

As part of a systematic investigation of the action of certain glycosides on enzyme systems we were interested in the effect of these drugs on the energy-yielding enzymatic reactions in connection with the therapeutic and toxic actions of these substances. The effect of digitoxin and ouabain *in vitro* on the ATP-ase activity of cardiac muscle was, therefore, studied, and the present preliminary report indicates that both of these drugs affect this enzymatic reaction.

ATP-ase activity was measured by the method of DuBois and Potter (*1*) using a Klett-Summerson colorimeter for phosphorus measurements. Normal Sprague-Dawley rats averaging 200 grams were employed. Aqueous solutions of ouabain were added to give a final concentration of 6×10^{-5} M, and 10 per cent alcoholic solutions of digitoxin were added in amounts sufficient to give a final concentration of 4.7×10^{-6} M.

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