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CONTENTS

Ferritin and Apoferritin

By Leonor Michaelis, *The Laboratories of The Rockefeller Institute for Medical Research, New York, New York*

Adsorption Analysis of Amino Acid Mixtures

By Arne Tiselius, *Upsala University, Upsala, Sweden*

Spread Monolayers of Protein

By Henry B. Bull, *Department of Chemistry, Northwestern University Medical School, Chicago, Illinois*

Films of Protein in Biological Processes

By Alexandre Rothen, *The Laboratories of The Rockefeller Institute for Medical Research, New York, New York*

The Chemical Determination of Proteins

By Paul L. Kirk, *The Division of Biochemistry, University of California Medical School, Berkeley, California*

Reactions of Native Proteins with Chemical Reagents

By Roger M. Herriott, *The Rockefeller Institute for Medical Research, Princeton, New Jersey*

The Amino Acid Requirements of Man

By Anthony A. Albanese, *Department of Pediatrics, New York University College of Medicine and Children's Medical Service, Bellevue Hospital, New York, New York*

The Use of Protein and Protein Hydrolyzates for Intravenous Alimentation

By Robert Elman, *Department of Surgery, Washington University and Barnes Hospital, Saint Louis, Missouri*

The Preparation and Criteria of Purity of the Amino Acids

By Max S. Dunn and Louis B. Rockland, *The Chemical Laboratory, University of California, Los Angeles, California*

The Plasma Proteins and Their Fractionation

By John T. Edsall, *Department of Physical Chemistry, Harvard Medical School, Boston, Massachusetts*

Transamination and the Integrative Functions of the Dicarboxylic Acids in Nitrogen Metabolism

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Contents

Introduction

Since the discovery of enzymatic transamination in 1937 (32, 33, 34) the development of this subject has been reviewed by Braunstein (23, 24), by Cohen (61, 62) and lately by Herbst (91). In the present article no full account is proposed of earlier findings and hypotheses in this field. It is timely, though, to summarize present knowledge of this metabolic process and to sift well-established facts from erroneous or doubtful conclusions due to imperfect experimental technique or to premature generalization. We also wish to correct certain misinterpretations and misquotations of our data, and to discuss differences of opinion.

To comply with the general purpose of this volume, attention is focused on the role of transamination in amino acid and protein metabolism, with a minimum of purely enzymological information. A rather extended discussion of the metabolic functions of dicarboxylic amino acids and their precursors is included to arrive at a clearer insight into the metabolic interrelations of transamination and its physiological significance, which are still imperfectly understood.

ABBREVIATIONS. The following abbreviations are used in the text:

GL — *l*-glutamic acid; AS — *l*-aspartic acid; AL — *l*-alanine; KG — *α*-ketoglutaric acid; OA — oxaloacetic acid; PU — pyruvic acid; gl-aph — glutamic aminopherase; as-aph — aspartic aminopherase; co-aph — coenzyme of aspartic aminopherase.

In the symbols denoting the more important transamination reactions, only the initial and final amino acids will be indicated, omitting the participating keto acids, *e.g.*,

GL⇌AL, for: *l*-glutamic acid+pyruvic acid⇌*l*-alanine + *α*-ketoglutaric acid;

AL⇌AS, for: *l*-alanine+oxaloacetic acid⇌*l*-aspartic acid+ pyruvic acid.

I. Remarks on Experimental Methods

In view of the lack of specific analytical methods for amino-dicarboxylic acids at that time, disappearance or formation of GL and AS was estimated, in our early studies on transamination, by determinations of NH_2-N in the aminodicarboxylic acid fraction precipitated according to Foreman. Although admittedly non-specific, this method is sufficiently reliable for roughly quantitative experiments of this type. We still use it for the rapid estimation of relative transamination rates in the absence of interfering substances. The method fails in the presence of certain other amino acids, *e.g.*, tyrosine, cystine, glycine, large amounts of leucine; its accuracy is, of course, insufficient for kinetic measurements or the detection of small changes of GL or AS. Cohen (56) should be thanked for developing a specific micromethod for GL, which enabled him to investigate the rapid reaction GL⇌AL and delimit the scope of the transamination process more correctly than we had done. Cohen (59, 60) used non-specific differential determinations of CO_2, liberated with chloramine T from AS (2 moles) and from other amino acids (1 mole), to estimate transformations of AS. In this laboratory, AS has been determined by the method of Fromageot and Heitz (79) with subtraction of the acetaldehyde equivalents of AL, lactic and malic acids. A strictly specific micromethod for AS has now been worked out by Braunstein and Nemchinskaya (38), based on exhaustive methylation of AS (Dakin (66)) and manometric determination of the resulting fumaric acid (113).

AL was formerly determined according to Kendall-Fürth or Fromageot and Heitz (79). Two highly sensitive and convenient micromethods for AL have been made available (Braunstein and Bychkov, 30), combining oxidation of AL to acetaldehyde either with isatin (resp. ninhydrin, *cf.*

Virtanen, 163) or according to Fromageot and Heitz, and specific photometry of $CH_3 \cdot CHO$ with *p*-hydroxydiphenyl. The isatin method requires removal of AS, the permanganate procedure eventually involves corrections for lactic acid and for threonine, because deaminated threonine is quantitatively oxidized to $CH_3 \cdot CHO$ by permanganate (30).

KG can be determined quantitatively by the method of Krebs (107) OA—colorimetrically after Straub (153), PU—with carboxylase or by the salicylic aldehyde reaction of Straub (153), *cf.* Braunstein (22).

Dependable analytic procedures are thus available at present for the major constituents of transamination systems. Caution is necessary, however, in the set-up and interpretation of experiments, to avoid fallacies due to side-reactions. Thus, transformations of preformed amino-N donors or acceptors in animal tissues have been mistaken for slight transamination of *d*-amino acids (75, 76, 26, 52) and of certain other added substrates (35, 36). GL formation from histidine and arginine, included by Cohen (58) in a table of transamination rates, is probably due to other transformations of these amino acids. Doubtful conclusions concerning transamination have been based on indiscriminate use of semiquantitative tests for rapidly metabolized substrates like OA and PU (40, 2, 3, 50, 52). *In every instance, two components, at least, of any transamination system should be determined.* We concur with Herbst (91) as to the desirability of identifying isolated reaction products whenever a new system is being explored.

II. Mechanism of the Transamination Reaction

1. *Non-Biological Transamination*

Deamination of amino acids by carbonyl compounds has long been known in organic chemistry. Classical instances are the Strecker reaction of amino acids with alloxan and similar reactions with *o*-quinones, isatin, ninhydrin, methylglyoxal, and the like. In such cases deamination is usually associated with decarboxylation of the amino acid. Condensation of the amino and carbonyl groups to yield Schiff's bases, and tautomeric transformation of these, are generally assumed as intermediary steps.

Formation of Schiff's bases is also postulated in the case of decarboxylation of keto acids by amino compounds (Langenbeck's artificial carboxylases). Recently bacterial amino acid decarboxylases have also been shown to contain an active carbonyl in their prosthetic component—phosphorylated pyridoxal (84).[1]

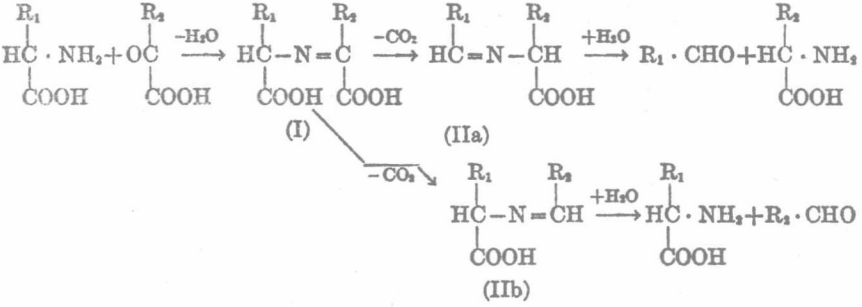
In 1934, Herbst and Engel (92) found that, in boiling aqueous solutions, the NH_2- group of α-amino acids is transferred to α-keto acids, a new

[1] In a preliminary note, published in January, 1945, F. Schlenk and E. E. Snell (145a) report evidence pointing to the possibility that the system: pyridoxal $\rightleftharpoons$ pyridoxamine may be involved in enzymatic transamination as an intermediary carrier of amino groups (see pp. 13–14).

amino acid being formed, while the original one is deaminated and decarboxylated to the corresponding aldehyde, *e.g.*:

phenylglycine + PU → AL + benzaldehyde + CO_2.

In many instances, the aldehyde $R_2 \cdot CHO$, corresponding to the original keto acid, is formed in addition to the aldehyde $R_1 \cdot CHO$, resulting from breakdown of the initial amino acid. The reaction mechanism is pictured as follows (Herbst, 91):



Scheme 1

Condensation of the substrates to Schiff's base (I) is followed by tautomeric shift of the double bond and simultaneous decarboxylation of the amino acid residue; hydrolysis of the aldimino-compound (IIa) to the end products ensues. Alternatively, the carboxyl adjacent to the double bond in Schiff's base (I) may be split off, and hydrolysis of the aldimino-compound (IIb) will result in recovery of the original amino acid and formation of the aldehyde R_2.CHO.

The rate of non-biological transamination is highest with aromatic α-amino acids or cystine, *i.e.*, with substrates inactive in enzymatic transamination (89). On the other hand, the dicarboxylic amino and α-keto acids react slowly in this model system (88, 91), whereas their participation is obligatory in enzymatic transamination (34, 23).

Non-biological transfer of amino groups has also been effected between α-amino acids and the carbonyl of α-ketoacylamino acids (Herbst and Shemin, 94); no well-established instance of reactions of this type in enzymatic systems is known as yet.

Recently, Brewer and Herbst (43) studied alkali-catalyzed transaminations in non-aqueous solution between the esters of amino and α-keto acids. These reactions are more closely analogous to enzymatic transamination, insofar as the amino acid residue is not decarboxylated and the reaction is strictly reversible.

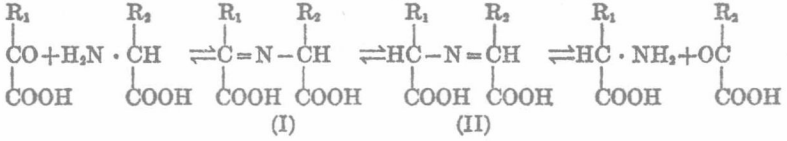
2. *Enzymatic Transamination*

The most extensively studied enzymatic transamination reactions of major biological importance in animal tissues are:

(1) GL+PU⇌AL+KG (Braunstein and Kritzmann, 33, 34).
(2) GL+OA⇌AS+KG (Szent-Györgyi and Banga, 10; Cohen 57).
(3) AS+PU⇌AL+OA (Karyagina, 99; Cohen and Hekhuis, 64).

These reactions have been shown to be reversible equilibrium reactions. Their configurational asymmetry has been demonstrated by the isolation and polarimetric investigation (contrary to the critical allusion of Herbst, 91) of pure *l*-GL (hydrochloride), $\alpha_D^{20} = +30.9°$, resp. $+32.3°$, in the reactions: *dl*-, resp. *l*-AL⇌GL (24, 26), and of pure toluenesulfonyl-*l*-AL, $\alpha_D^{16} = +7.5°$, in the reaction AS⇌AL (99).

The reaction scheme of enzymatic transamination assumed by Braunstein and Kritzman (34) is analogous to the mechanism postulated by Herbst for the non-biological reaction (92, 91):



Scheme 2

The intramolecular oxidoreduction, or reversible prototropic change in the methylene-azomethine bridge (interconversion of Schiff's bases I and II) is thought to be the enzymatically catalyzed step.

Direct evidence for the spontaneous condensation of amino and keto acids in aqueous media was provided by Knoop and Martius (100) in their synthesis of a diastereoisomer of octopine (*cf.* Herbst, 91) by catalytic hydrogenation of a solution of arginine and PU. However, arginine is inactive in enzymatic transamination (24). Karrer and associates (96) maintained that octopine is oxidized in minced liver tissue, and suggested that transamination might involve intermediary hydrogenation of Schiff's base I to an $\alpha\alpha_1$-iminodicarboxylic acid (octopine analog) and subsequent dehydrogenation to Schiff's base II. They also assumed that $\alpha\alpha_1$-iminodipropionic acid and its homologs may act as intermediates in oxidative deamination of *l*-amino acids (97), but negative results of later more careful experiments by Karrer and Appenzeller (98) caused them to abandon this hypothesis.

3. *Transamination Studies with Deuterium*

The mechanism of hydrogen transfer in enzymatic transamination has been studied by Konikova, Kritzmann and Teiss (102) ine xperiments with α-deuterio-alanine. In the presence of crude gl-aph and of KG the greater part of the deuterium contained in labeled AL passes into the water; the newly formed GL contains practically no excess of D. No significant loss of