COMPREHENSIVE BIOCHEMISTRY

EDITED BY

MARCEL FLORKIN

AND

ELMER H. STOTZ

VOLUME 15

GROUP-TRANSFER REACTIONS

COMPREHENSIVE BIOCHEMISTRY

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SECTION I (VOLUMES 1–4)

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SECTION II (VOLUMES 5–11)

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GENERAL PREFACE

The Editors are keenly aware that the literature of Biochemistry is already very large, in fact so widespread that it is increasingly difficult to assemble the most pertinent material in a given area. Beyond the ordinary textbook the subject matter of the rapidly expanding knowledge of biochemistry is spread among innumerable journals, monographs, and series of reviews. The Editors believe that there is a real place for an advanced treatise in biochemistry which assembles the principal areas of the subject in a single set of books.

It would be ideal if an individual or small group of biochemists could produce such an advanced treatise, and within the time to keep reasonably abreast of rapid advances, but this is at least difficult if not impossible. Instead, the Editors with the advice of the Advisory Board, have assembled what they consider the best possible sequence of chapters written by competent authors; they must take the responsibility for inevitable gaps of subject matter and duplication which may result from this procedure.

Most evident to the modern biochemist, apart from the body of knowledge of the chemistry and metabolism of biological substances, is the extent to which he must draw from recent concepts of physical and organic chemistry, and in turn project into the vast field of biology. Thus in the organization of Comprehensive Biochemistry, the middle three sections, Chemistry of Biological Compounds, Biochemical Reaction Mechanisms, and Metabolism may be considered classical biochemistry, while the first and last sections provide selected material on the origins and projections of the subject.

It is hoped that sub-division of the sections into bound volumes will not only be convenient, but will find favour among students concerned with specialized areas, and will permit easier future revisions of the individual volumes. Toward the latter end particularly, the Editors will welcome all comments in their effort to produce a useful and efficient source of biochemical knowledge.

Liège/Rochester

M. Florkin
E. H. Stotz

PREFACE TO SECTION III

(VOLUMES [illegible]-16)

Following Section II of Comprehensive Biochemistry on the Chemistry of Biological Compounds, and preceding sections on Metabolism and Chemical Biology, Section III is devoted primarily to Enzymes. Recognizing the encyclopedic nature of any effort to provide even a minimal treatment of all known enzymes, the Editors have chosen instead to select examples from modern enzymology in which advances in reaction mechanisms have been made. Certainly a well-established biochemical reaction mechanism is the carrier function of coenzymes which serve as the prosthetic groups of enzymes, and Section III has a primary purpose of providing treatment of both the chemistry and function of the coenzymes. Other chapters, however, treat thermodynamic and kinetic aspects of enzyme catalysis, hydrolytic enzymes displaying "active center" characteristics, and chelation and stereochemical considerations in enzyme catalysis. A considerable portion of the Section deals with biological oxidation mechanisms. Finally, Section III would seem incomplete without inclusion of the recommendations of the Enzyme Commission of the International Union of Biochemistry and the classified list of Enzymes.

Liège/Rochester — M. FLORKIN
January 1964 — E. H. STOTZ

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Chapter I

Biological Transmethylation, Methyl-Group Neogenesis and Other "One-Carbon" Metabolic Reactions Dependent Upon Tetrahydrofolic Acid

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This Chapter will deal with the enzymology of one-carbon fragments at the methyl, hydroxymethyl and formyl levels of oxidation. Emphasis will be given to the synthesis and transfer of the methyl group. One-carbon fragments are formed *de novo* from formaldehyde or formate, or generated metabolically from a variety of precursors. Whatever their origin, these one-carbon units may be converted from one oxidation level to another and transferred enzymatically to a variety of acceptors. The metabolic role of folic acid will be discussed but only insofar as it is involved with the pathways mentioned. Space precludes any attempt to cover other aspects of folic acid metabolism such as its biosynthesis, interconversion of the several forms, or its recently established role in the hydroxylation of phenylalanine to tyrosine[1]. We will not deal with one-carbon metabolism at the methane or carbon dioxide levels, or with those one-carbon processes in which FH_4 is not involved* (listed, for instance, by Sakami[2]).

A. FORMYL AND HYDROXYMETHYL GROUP METABOLISM

1. General background

The early developments which led to the concept that folic acid derivatives are involved in the enzymatic transfers of one-carbon units at the oxidation

* The following abbreviations are used: FH_4, tetrahydrofolic acid; f^5FH_4 and $f^{10}FH_4$, N^5- and N^{10}-formyltetrahydrofolic acid; $f^{5-10}FH_4$, N^5,N^{10}-methenyltetrahydrofolic acid; $h^{5-10}FH_4$, N^5,N^{10}-methylenetetrahydrofolic acid; m^5FH_4, N^5-methyltetrahydrofolic acid; AMe, (–)-*S*-adenosyl-L-methionine.

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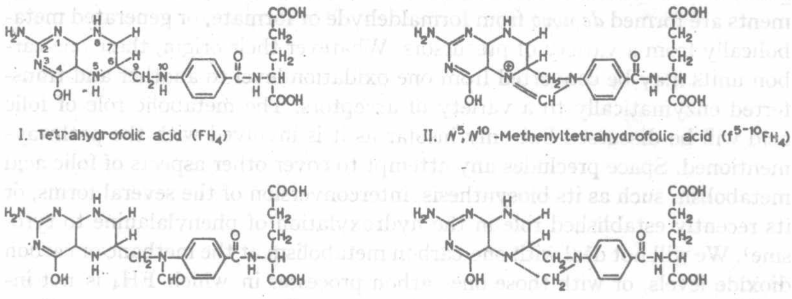
level of formate and formaldehyde were reviewed by Huennekens and Osborn[3]. As a result of a variety of experimental approaches involving the use of tracer techniques, nutritional studies, the isolation of various naturally occurring forms of folic acid, and finally, the isolation and study of individual enzymes, the relationships between formate, formaldehyde and the one-carbon unit in other metabolites have become clear. A general description of one-carbon metabolism at the enzymatic level is provided by the schematic equations A, B and C (see also Huennekens[3]):

$$\text{D-X} + \text{C} \rightleftharpoons \text{CX} + \text{D} \qquad \text{(A)}$$

$$\text{CX} + \text{A} \rightleftharpoons \text{A-X} + \text{C} \qquad \text{(B)}$$

$$\text{DX} + \text{A} \rightleftharpoons \text{AX} + \text{D} \qquad \text{(C)}$$

In these equations X represents the formaldehyde or formate group, C the folic acid coenzyme, D the donor molecule containing a potential one-carbon unit (serine, purine, histidine, etc.) and A an acceptor molecule (glycine, carboxamide ribotide, etc.). It is clear that in these reactions the folic acid coenzyme acts as a carrier of the one-carbon group which is being transferred. Some of the pertinent structures are shown in Fig. 1.



I. Tetrahydrofolic acid (FH_4) II. N^5,N^{10}-Methenyltetrahydrofolic acid ($f^{5-10}FH_4$)

III. N^{10}-Formyltetrahydrofolic acid ($f^{10}FH_4$) IV. N^5,N^{10}-Methylenetetrahydrofolic acid ($h^{5-10}FH_4$)

Fig. 1. Structures of some folic acid compounds.

An outline of the best known metabolic sequences in which folic acid participates as a one-carbon carrier is shown in Fig. 2. For purposes of orientation, it may be worthwhile briefly to discuss the reactions shown on this metabolic map.

The one-carbon bearing derivatives of FH_4 arise in two ways: (*a*) through attachment of free formic acid (reaction 1) or of free formaldehyde (reaction 2) under the influence of the appropriate activating enzymes; and (*b*) through

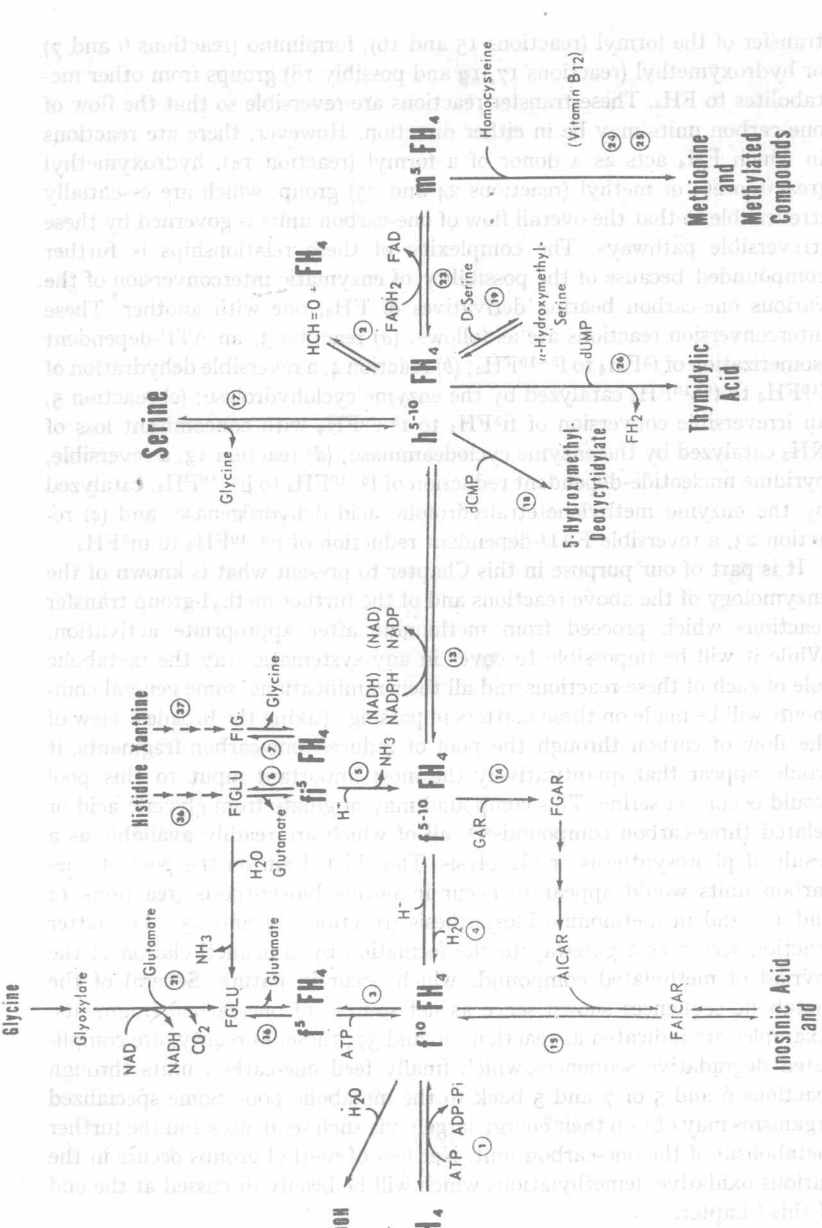


Fig. 2. The metabolic relationships of tetrahydrofolic acid.

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transfer of the formyl (reactions 15 and 16), formimino (reactions 6 and 7) or hydroxymethyl (reactions 17, 19 and possibly 18) groups from other metabolites to FH_4. These transfer reactions are reversible so that the flow of one-carbon units may be in either direction. However, there are reactions in which FH_4 acts as a donor of a formyl (reaction 14), hydroxymethyl (reaction 26) or methyl (reactions 24 and 25) group, which are essentially irreversible so that the overall flow of one-carbon units is governed by these irreversible pathways. The complexity of these relationships is further compounded because of the possibility of enzymatic interconversion of the various one-carbon bearing derivatives of FH_4, one with another. These interconversion reactions are as follows: (*a*) reaction 3, an ATP-dependent isomerization of f^5FH_4 to $f^{5-10}FH_4$; (*b*) reaction 4, a reversible dehydration of $f^{10}FH_4$ to $f^{5-10}FH_4$ catalyzed by the enzyme cyclohydrolase; (*c*) reaction 5, an irreversible conversion of fi^5FH_4 to $f^{5-10}FH_4$ with concomitant loss of NH_3 catalyzed by the enzyme cyclodeaminase; (*d*) reaction 13, a reversible, pyridine nucleotide-dependent reduction of $f^{5-10}FH_4$ to $h^{5-10}FH_4$, catalyzed by the enzyme methylenetetrahydrofolic acid dehydrogenase; and (*e*) reaction 23, a reversible FAD-dependent reduction of $h^{5-10}FH_4$ to m^5FH_4.

It is part of our purpose in this Chapter to present what is known of the enzymology of the above reactions and of the further methyl-group transfer reactions which proceed from methionine after appropriate activation. While it will be impossible to cover in any systematic way the metabolic role of each of these reactions and all their ramifications, some general comments will be made on these matters in passing. Taking the broadest view of the flow of carbon through the pool of reduced one-carbon fragments, it would appear that quantitatively the most important input to this pool would occur via serine. This compound may originate from glyceric acid or related three-carbon compounds[4,5], all of which are readily available as a result of photosynthesis or glycolysis. The chief drain on the pool of one-carbon units would appear to occur in purine biosynthesis (reactions 14 and 15) and in methionine biosynthesis (reactions 24 and 25). The latter reaction serves as a gateway to the formation by transmethylation of the myriad of methylated compounds which occur in nature. Several of the metabolic sequences shown serve as net sources of one-carbon fragments. Examples are indicated as reactions 36 and 37; these, in reality, are complicated degradative sequences which finally feed one-carbon units through reactions 6 and 5 or 7 and 5 back to the metabolic pool. Some specialized organisms may obtain their energy largely via such sequences and the further metabolism of the one-carbon unit. Net loss of methyl groups occurs in the various oxidative demethylations which will be briefly discussed at the end of this Chapter.

We will now proceed with the presentation of the enzymology of the individual reactions. A detailed discussion of the mechanisms of some of these reactions has been presented by Huennekens *et al.*[3] and by Jaenicke[6]. The latter author also discusses the use of certain *N,N'*-diarylethylenediamine compounds in model experiments to elucidate the chemistry of atoms 5, 6, 9 and 10 of tetrahydrofolic acid, the atoms comprising the region of the coenzyme which combines with one-carbon units.

2. Activation reactions

(a) *Formate activation*

The mechanism of this reaction has been intensively investigated, but at present no single mechanism has been fully proven. Investigators using formate-activating enzymes from different sources have found many features

$$HCOOH + ATP + FH_4 \rightleftharpoons N^{10}\text{-formyltetrahydrofolate} + ADP + P_i \qquad (1)$$

of the reaction to be similar. However, a major point still unresolved is whether a phosphorylated form of FH_4 is intermediate in the reaction. Three chief lines of evidence bear on this point: (*a*) The formation of ADP when ATP is incubated with enzyme in the presence of FH_4, (*b*) the requirements for an ATP–ADP exchange, (*c*) the requirements for a formate–formyl–FH_4 exchange. Since the findings in the three most thoroughly studied enzymes differ in these areas, different detailed schemes have been postulated for the reaction and it is necessary to consider these three systems separately.

(*i*) *Clostridium cylindrosporum enzyme*

The most highly purified formate-activating enzyme is the crystalline preparation from *Clostridium cylindrosporum*[7]. As a result of their studies with the enzyme, Himes and Rabinowitz[8] have suggested that the reaction proceeds by a "concerted" mechanism in which the three substrates and the enzyme interact to produce the three products ($f^{10}FH_4$, ADP and P_i) without participation of free activated intermediates or of enzyme–phosphate complexes.

Evidence against the formation of a free phosphorylated intermediate is provided by the finding that ADP was not formed when the enzyme was incubated with ATP and with only one of the two other substrates, formate or FH_4. Under these conditions formation of ADP was not detected even in the presence of an enzymatic system which would remove any ADP formed and so "pull" the reaction.

The enzyme catalyzes a relatively slow ATP–[^{32}P]ADP exchange. Neither formate nor FH_4 alone alters the rate of this exchange, while addition of both formate and FH_4 increases the rate 20-fold. The lack of stimulation of the

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ATP–ADP exchange by either formate of FH_4 alone is further evidence against formation of a phosphorylated derivative of either of these compounds as a first step in the reaction. The stimulatory effect by formate and FH_4 together suggests that E ~ P is not formed as a first step because in such a case formate and FH_4 together would depress the ATP–ADP exchange through removal of E ~ P.

The crystalline enzyme catalyzes an ATP–$^{32}P_i$ exchange. The fact that *both* formate and FH_4 are required for this reaction suggests that an ADP anhydride derivative of either of these substrates is not formed as a first step. Further, the finding that during the reaction one oxygen atom is transferred from formate to the P_i formed in the reaction rules out the intermediate formation of the anhydride, formyl–ADP.

Together, these findings led Himes and Rabinowitz to propose the "concerted" mechanism shown in Fig. 3. This mechanism is consistent with all the

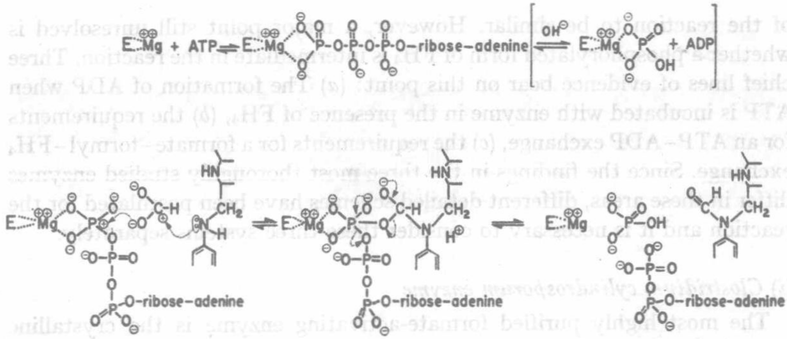

Fig. 3. The mechanism of formate activation proposed by Himes and Rabinowitz for the *Clostridial* enzyme.

findings listed. It is also consistent with the observations that $f^{10}FH_4$ is arsenolyzed by the enzyme, but only in the presence of ADP, and that the formate–$f^{10}FH_4$ exchange catalyzed by the enzyme is dependent upon ADP. This scheme, however, does not account for the slow ATP–ADP exchange catalyzed by the crystalline enzyme in the absence of both formate and FH_4. Contamination with enzymes known to catalyze an ADP–ATP exchange such as adenylate kinase, nucleoside diphosphokinase, or ATPase was shown not to account for this exchange, but the possibility can, of course, not be eliminated that a contaminant might be responsible for this exchange. In this connection, it is of interest that heat treatment and treatment with *p*-mercuribenzoate results in greater losses in the overall synthetase activity than in ATP–ADP exchange. Himes and Rabinowitz suggest on the basis of this line of