sulting in well-defined aggregations of species into clearly bounded and readily pigeonholed units as objective natural entities. Much recent evidence points toward the concept of communities as an ordered pattern of species, individually distributed in space and time and most effectively considered in terms of orders and gradients.

References and Notes

1. A. Kerner, *The Plant Life of the Danube Basin* (1863). Translated by H. S. Conard as *The Background of Plant Ecology* (Iowa State College Press, Ames, 1951).
2. P. Whitford and K. Whitford, *Sci. Monthly* 73, 291 (1951).
3. S. A. Cain, *Am. Midland Naturalist* 21, 27 (1939).
4. J. E. Weaver and F. E. Clements, *Plant Ecology* (McGraw-Hill, New York, 1938).
5. H. S. Conard, *Am. Midland Naturalist* 16, 433 (1935).
6. C. Flauhault and C. Schröter, *Proc. Intern. Botan. Congr. 3rd Congr.* (1910).
7. H. S. Conard, *Am. Midland Naturalist* 21, 1 (1939).
8. H. A. Gleason, *Bull. Torrey Botan. Club* 53, 7 (1926).
9. ———, *Am. Midland Naturalist* 21, 92 (1939).
10. W. D. Billings, *ibid.* 42, 87 (1949).
11. J. T. Curtis and R. P. McIntosh, *Ecology* 32, 476 (1951).
12. J. T. Curtis, *ibid.* 36, 558 (1955).
13. R. H. Whittaker, *Ecol. Monographs* 26, 1 (1956).
14. J. R. Bray, *Ecology* 37, 21 (1956).
15. M. Gilbert and J. T. Curtis, *Trans. Wisconsin Acad. Sci.* 42, 183 (1953).
16. W. E. Randall, *Ecology* 34, 544 (1953).
17. H. D. Tresner, M. P. Backus, J. T. Curtis, *Mycologia* 46, 314 (1954).
18. M. E. Hale, Jr., *Ecology* 36, 45 (1955).
19. R. E. Bond, *Bull. Ecol. Soc. Am.* 37, 74 (1956).
20. R. P. McIntosh, *Ecology* 38, 29 (1957).
21. L. C. Cole, *ibid.* 30, 411 (1949).
22. E. S. Dyksterhuis, *J. Range Management* 2, 104 (1949).
23. J. Braun-Blaunquet, *Plant Sociology: The Study of Plant Communities.* Translated by G. D. Fuller and H. S. Conard (McGraw-Hill, New York, 1932).
24. D. W. Goodall, *Angew. Pflanzensoz.* 1, 168 (1954).

Folic Acid Coenzymes

Metabolic reactions involving "active formate" and "active formaldehyde" are surveyed.

F. M. Huennekens, M. J. Osborn, H. R. Whiteley

For a great variety of cells of animal, plant, and microbial origin, the vitamin, folic acid, serves as a growth factor by controlling the metabolism of the one-carbon (C_1) compounds, formate and formaldehyde. These C_1 compounds serve as building blocks in the biosynthesis of purines, certain pyrimidines, and certain amino acids. An increased capacity to incorporate formate into cellular nucleic acids (*1*), and also an elevated level of folic acid (*2*) and its coenzyme forms (*3*), has been found to characterize at least one type of abnormal growth, namely that of the leukemic white cell.

A description of C_1 metabolism is provided by the following generalized equations for the mobilization of X, the formaldehyde or formate group,

$$D—X + C \rightleftharpoons C—X + D \qquad (1a)$$

$$C—X + A \rightleftharpoons A—X + C \qquad (1b)$$

In these equations, C is the folic acid coenzyme, D is a donor molecule containing a potential C_1 unit (serine, purine, histidine, and so on), and A is an acceptor molecule (glycine, carboxamide ribotide, and so on). When the C_1 unit is bound to the folic acid coenzyme ($C—X$), the complex may be considered as "active formate" or "active formaldehyde," respectively; the latter complex is more frequently referred to as "active hydroxymethyl." The analogy between these complexes and the well-known "active acetate" (acetyl coenzyme A) is apparent.

Although the early recognition of various metabolites as donors and acceptors of formyl and hydroxymethyl units was achieved by tracer and nutritional studies (*4, 5*), recent work has been directed toward studies with isolated enzyme systems, which permit a greater degree of understanding of reaction mechanisms. Study of the detailed mechanisms of the various reactions, represented by Eqs. 1a and 1b, has been hampered by the multiplicity of the C_1 fragments (formyl, hydroxymethyl, and in some instances, methyl) and by uncertainty concerning the chemical structure of the intermediates, $C—X$, since, as is discussed in subsequent paragraphs, folic acid has several potential sites of attachment for a C_1 group.

In this article (*6*) we shall attempt to summarize the current information regarding the nature of "active formyl" and "active hydroxymethyl" complexes and to survey the various metabolic reactions in which these compounds participate (*7*).

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Reactions Involving "Active Formaldehyde"

Serine hydroxymethylase. Numerous investigators (summarized in reference *8*) have studied the enzymatic interconversion of serine and glycine. In early work (*9*), in which tissue slices or intact animals were used, sensitive tracer techniques were required to detect the small conversions. More recently, larger conversions have been achieved by use of purified enzymes from avian and mammalian livers (*8*; *10–13*).

The over-all reaction (Eq. 2) for the biosynthesis of serine from glycine and HCHO

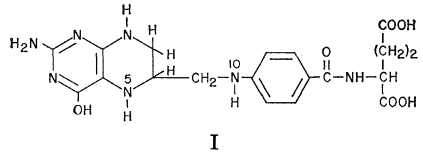
$$\text{HCHO} + \text{glycine} \rightleftharpoons \text{serine} \qquad (2)$$

is actually the sum of two separate reactions

$$\text{HCHO} + \text{FH}_4 \rightleftharpoons \text{hFH}_4 \qquad (2a)$$

$$\text{hFH}_4 + \text{glycine} \rightleftharpoons \text{serine} + \text{FH}_4 \qquad (2b)$$

where FH_4 is the abbreviation (*14*) for 5,6,7,8-tetrahydrofolic acid (I)



I

the coenzyme form of folic acid, and hFH_4 symbolizes hydroxymethyl FH_4, without specifying the position of the C_1 group on the folic acid coenzyme.

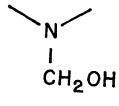
Reactions 2a and 2b are catalyzed by the formaldehyde-activating enzyme and serine hydroxymethylase [or serine aldolase (*11*)], respectively.

In the over-all reaction (Eq. 2) a requirement for pyridoxal phosphate has been observed in all systems studied (*8*; *10–13*; *16*); an additional requirement for Mn^{++} has been demonstrated only with a bacterial enzyme (*15*). The site of action of both cofactors is undoubtedly in reaction 2b, as would be anticipated from the chemical studies of Snell and his co-workers (*16*).

Formaldehyde-activating enzyme. Since spectroscopic evidence indicates that HCHO and FH_4 interact rapidly in the absence of any enzyme to form one or more complexes, it was not immediately clear whether reaction 2a was enzyme-catalyzed or not. However, during purification of the enzyme system that catalyzes reaction 2, it was found that the enzymes for reactions 2a and 2b could be partially separated (*13*). The formaldehyde-activating enzyme can be assayed in the following manner. When FH_4 ($\lambda_{max.}$ at 300 mμ, $\varepsilon \cong 22 \times 10^6$ cm²/mole) is mixed with a slight excess of HCHO at *p*H 7, no reaction occurs. When the purified activating enzyme is added, the spectral peak shifts to 294 mμ, and ε increases to 26×10^6 cm²/mole. This shift is due to the formation of hFH_4 according to Eq. 2a. When hFH_4 is synthesized enzymatically, it is a reasonably stable substance and can be separated from unreacted FH_4 by column chromatography or by paper chromatography (*17*).

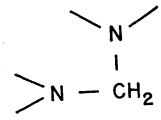
The authenticity of hFH_4 is established by the following enzymatic assays. (i) In the presence of serine hydroxymethylase and glycine, hFH_4 disappears and FH_4 reappears (see reactions 2b). (ii) In the presence of the hFH_4 dehydrogenase (see below) and TPN, hFH_4 is oxidized to formyl FH_4, and an equivalent amount of TPNH is formed. Non-enzymatically, hFH_4 may be converted to FH_4 by the addition of hydroxylamine in acid; in a basic medium the material is stable to H_2NOH.

Structure of "active formaldehyde." Formaldehyde is known to react with —NH_2 or —SH groups, forming equilibrium adducts of the type represented by II.



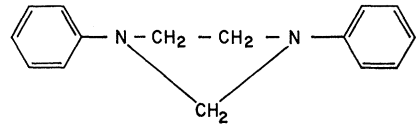
II

When a second ligand is possible with the bound HCHO, as in III,



III

much more stable complexes are formed, especially if both ligands are on the same molecule. An example of this is the reaction of HCHO with cysteine to form the stable heterocycle, thiazolidine carboxylic acid. An important clue to the structure of hFH_4, was provided by Blakley (*18*), who prepared the HCHO adduct (IV) of N,N-diphenyl ethylenediamine:



IV

Structure IV is quite stable, and, like thiazolidine carboxylic acid, it does not release its bound HCHO to aldehyde-binding agents such as chromotropic acid and acetyl acetone. Although hFH_4 in acid behaves like a type II adduct in releasing HCHO, its stability in base strongly suggests a type III structure, very probably $h^{5\text{-}10}FH_4$ (*19*). Confirmatory evidence for this proposed structure comes from a study of the substrate specificity of the hFH_4 dehydrogenase, as described below.

While HCHO and FH_4 react rapidly in the absence of enzyme to form a product which is active in systems requiring hFH_4, the reaction is not confined to a single product as in the enzymatic synthesis. Various spectroscopic complexes, whose $\lambda_{max.}$ range between 290 and 300 mμ, are formed when increasing amounts of HCHO are added to FH_4. This result is not unexpected, for FH_4 contains several potential sites, namely, the N^3, N^5, N^8, and N^{10} positions, for binding HCHO.

Reactions Involving "Active Formate"

Hydroxymethyl tetrahydrofolic acid (hFH_4) dehydrogenase. The metabolic equivalence of formyl and hydroxymethyl donors in early tracer studies of C_1 reactions (that is, the labeling of the C-2 position of the purine ring both by formate and by the β-carbon atom of serine) presupposed the existence of a system for the interconversion of the two oxidation states. A TPN-linked dehydrogenase, carrying out this interconversion, was first described by Jaenicke (*20*) and by Greenberg *et al.* (*21*) and was later obtained in purified form in our laboratory (*22*). The over-all reaction is shown in Eq. 3.

$$hFH_4 + TPN^+ \rightleftharpoons fFH_4 + TPNH + H^+. \quad (3)$$

For the moment, the precise nature of hFH_4 and fFH_4 is not specified. Reaction 3 is best studied in conjunction with reactions 2b or 2a, serine or HCHO being used to generate hFH_4. When crude dehydrogenase preparations are used, the accumulated reaction product, fFH_4, is the N^{10}-isomer, $f^{10}FH_4$. However, when dehydrogenase preparations are freed from the enzyme, cyclohydrolase (*23*), which carries out reaction 4,

$$(f^{5\text{-}10}FH_4)^+ + H_2O \rightleftharpoons f^{10}FH_4 + H^+ \quad (4)$$

the end product of reaction 3 is $f^{5\text{-}10}FH_4$. With purified dehydrogenase preparations, $f^{5\text{-}10}FH_4$, but not $f^{10}FH_4$ or f^5FH_4, serves as a substrate for reaction 3 in reverse. Similarly, $f^{5\text{-}10}FH_4$ (but not f^5FH_4 or $f^{10}FH_4$) can be reduced quantitatively with borohydride, and the product is identical to authentic hFH_4 as measured by absorption spectrum and enzymatic assays. The experiments cited above provide support for the hypothesis that both hFH_4 and fFH_4 in reaction 3 are the N^5, N^{10}-bridge compounds. Greenberg and Jaenicke (*7*) and Kisliuk (*24*) have also suggested that hFH_4 is $h^{5\text{-}10}FH_4$.

The equilibrium constant for reaction 3 is near unity at *p*H 8.0 (the *p*H optimum for the enzyme), and the extent of the reaction is dependent, of course, upon H^+. This can be demonstrated by starting with $f^{10}FH_4$ (in the presence of cyclohydrolase) and following the disappearance of TPNH spectrophotometrically at 340 mμ. After the equilibrium position has been established, the reaction may be driven further to the left, as evidenced by further disappearance of TPNH, by the addition of H^+, or by the addition of glycine (to "trap" hFH_4 via reaction 2b).

In addition to facilitating the interconversion of C_1 units at the levels of formyl and hydroxymethyl, the dehydrogenase can be used as a spectroscopic "marker" for following a variety of re-

actions (see Fig. 1) that involve hFH_4 or fFH_4. Furthermore, the equilibrium of the dehydrogenase itself (and, of course, any coupled system involving the dehydrogenase) can be manipulated in either direction—for example, by trapping TPNH via the glutathione reductase system or by trapping TPN with glucose-6-phosphate dehydrogenase.

N^{10}-formyl tetrahydrofolic acid ($f^{10}FH_4$) deacylase. Studies with the dehydrogenase in beef and pigeon liver preparations led to the discovery of a deacylase (*25*) specific for $f^{10}FH_4$:

$$f^{10}FH_4 + H_2O \rightarrow HCOOH + FH_4 \quad (5)$$

This enzyme is present in beef liver preparations but absent from pigeon liver preparations. When the dehydrogenase was studied with the latter tissue, the amounts of TPNH and $f^{10}FH_4$ formed during the reaction were equal. With the former tissue, the amount of accumulated $f^{10}FH_4$ was much less than the TPNH because of the concomitant action of the deacylase. The deacylase has been partially purified from beef liver preparations; it can be added to pigeon liver dehydrogenase to produce the beef liver pattern (that is, TPNH » $f^{10}FH_4$). A more simplified assay for the deacylase consists of incubating $f^{10}FH_4$ (absorption maximum at 258 mμ) directly with the enzyme and measuring the increase in optical density at 300 mμ as FH_4 is produced. Since small quantities of formate are difficult to estimate, the stoichiometry of reaction 5 has been studied with respect to FH_4. 5,6,7,8-Tetrahydrofolic acid (FH_4) has been identified by its activity with the formate-activating enzyme (see below) and by its activity in the coupled hydroxymethylase-dehydrogenase system:

$$\text{Serine} + FH_4 + TPN^+ \rightleftharpoons \text{glycine} + f^{10}FH_4 + TPNH + H^+ \quad (6)$$

The deacylase for $f^{10}FH_4$ probably serves to regenerate FH_4 in the absence of actively functioning acceptors for "active formyl," much as the deacylase for acetyl coenzyme A provides a means for regenerating coenzyme A in the absence of acceptors for "active acetate."

Formate-activating enzyme. Since free formate has long been known to enter metabolic systems involving "active formyl" and "active hydroxymethyl" groups, it was anticipated that there would be a formate-activating enzyme. Formate activation was first reported (*21, 26*) in connection with studies of the biosynthesis of purines, and the following stoichiometry was proposed:

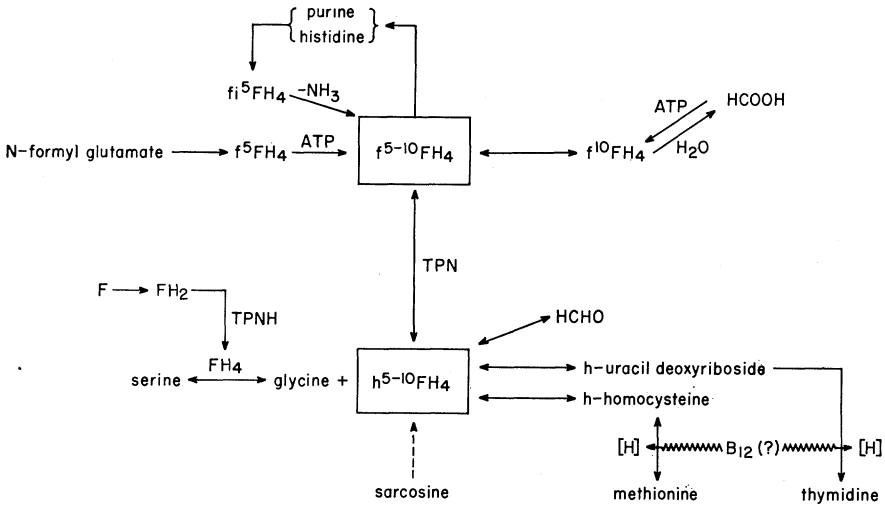


Fig. 1. Metabolic interrelationships of active C_1 units.

$$HCOOH + ATP + FH_4 \xrightarrow{Mg^{++}} f^{10}FH_4 + ADP + P_i \quad (7)$$

The formation of ADP and P_i as end products makes this reaction similar to the activation of glutamate or succinate. In contrast, the activation of other carboxylic acids, such as acetate or butyrate, produces AMP and PP as end products. Using a highly purified activating enzyme from *Micrococcus aerogenes,* we have shown (*27*) that the sequence in reaction 7 is

$$ATP + FH_4 \rightleftharpoons ADP + pFH_4 \quad (7a)$$
$$pFH_4 + HCOOH \rightleftharpoons f^{10}FH_4 + P_i \quad (7b)$$

The mechanism thus resembles that proposed for the formation of succinyl-CoA, where S-phosphoryl CoA is the analogous intermediate (*28*). Spectroscopic evidence has been adduced for reaction 7a; when enzyme and ATP are added to FH_4, the peak at 300 mμ shifts to shorter wavelengths, and ε decreases about 15 percent. "pFH_4," labeled with P^{32}, has also been isolated from reaction mixtures by use of ATP^{32}. Reaction 7a is also followed conveniently by measuring the liberated ADP with the coupled system employing pyruvic kinase and lactic dehydrogenase:

$$\text{ADP} + \text{phospho-enol pyruvate} \rightleftharpoons ATP + \text{pyruvate} \quad (8)$$

$$\text{Pyruvate} + DPNH + H^+ \rightleftharpoons \text{lactate} + DPN^+ \quad (9)$$

The observed reaction product in Eq. 7 is $f^{10}FH_4$. Since the purified bacterial enzyme contains no cyclohydrolase, it seems probable that $f^{5\text{-}10}FH_4$ is not an intermediate in this reaction. Consequently, it would seem most likely that the intermediate in reactions 7a and 7b, pFH_4, is N^{10}-phosphoryl FH_4 ($p^{10}FH_4$ in our symbols), but the verification of this assumption must await the unambiguous chemical synthesis of this compound.

N^5-formyl tetrahydrofolic acid (f^5FH_4) isomerase. In all of the studies mentioned above, $f^{10}FH_4$ or $f^{5\text{-}10}FH_4$ has been involved as the "active formyl" group. The question may be raised, therefore, about the relationship of the classical formyl isomer, f^5FH_4 (folinic acid), to "active formate." Except for the formylation of glutamate (*29*), where f^5FH_4, rather than $f^{10}FH_4$ or $f^{5\text{-}10}FH_4$, is the formyl donor, folinic acid is curiously inert metabolically. There is, however, an enzyme system (*30*) present in many tissues which brings about the apparently unidirectional isomerization of f^5FH_4 to $f^{10}FH_4$ via the following reaction:

$$f^5FH_4 + ATP \xrightarrow{Mg^{++}} f^{10}FH_4 + ADP + P_i \quad (10)$$

Recent studies by Peters and Greenberg (*31*) have suggested that AMP and PP, rather than ADP and P_i, are the reaction products. It has been established (*31, 32*) that reaction 10 in certain tissues may be separated into two separate steps:

$$f^5FH_4 + ATP \rightarrow f^{5\text{-}10}FH_4 + ADP + P_i \quad (10a)$$
$$\text{(or AMP + PP)}$$

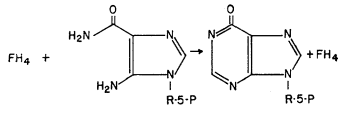
and

$$f^{5\text{-}10}FH_4 + H_2O \rightarrow f^{10}FH_4 \quad (10b)$$

Reaction 10b is catalyzed by cyclohydrolase, while the enzyme responsible for reaction 10a may be termed *cyclodehydrase,* by analogy to the *cyclodeaminase* reaction studied by Rabinowitz and Pricer (*23*):

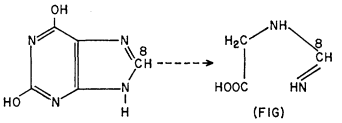
$$fi^5FH_4 \rightarrow f^{5\text{-}10}FH_4 + NH_3 \quad (11)$$

Biosynthesis and breakdown of purine and histidine. The laboratories of Buchanan (*33*), and G. R. Greenberg (*26*) have shown that both the 2- and 8-carbon atoms of the purine ring arise by the action of a specific transformylase with $f^{10}FH_4$ (or $f^{5\text{-}10}FH_4$) as the formyl donor.



(12)

In contrast, the *breakdown* of purines, at least in certain bacterial systems, is much more complicated. Cleavage of xanthine gives rise to CO_2 from the C-2 position, but the C-8 carbon emerges as the formimino group, H—C=NH, of formiminoglycine (FIG) (*23, 34*):



(13)

Formiminoglycine is then decomposed according to the sequence:

$$FIG + FH_4 \rightarrow fi^5FH_4 + \text{glycine} \quad (14)$$

followed by reactions 11 and 10b. Similarly, in mammalian liver (*35*), histidine is degraded to the analogous intermediate, formiminoglutamic acid (FIGlu), which, in turn, is metabolized by a series of reactions analogous to 14, 11, and 10b. The pathway of histidine biosynthesis has not yet been elucidated fully, although the involvement of a formyl or formimino group has been demonstrated.

Formate exchange into pyruvate. Recent reports (*36*) indicate that the exchange of $HC^{14}OOH$ into pyruvate by crude extracts of *Escherichia coli* is stimulated by the addition of FH_4. Similar results have been obtained with extracts of *Micrococcus aerogenes* and *Micrococcus lactilyticus* (*37*). However, the identity of the C_1 complex participating in the exchange reaction is not known, and the mechanism of the reaction has not been established. In the anaerobic micrococci the exchange reaction does *not* occur via serine as an intermediate—that is, by synthesis of serine from formate and endogenous glycine followed by deamination to pyruvate.

Structure of "active formate." Because of its early recognition, folinic acid (f^5FH_4) was assumed to be the "active formate" in C_1 metabolism. Subsequent work with purified enzyme systems, however, has revealed that f^5FH_4 functions only in the formylation of glutamate (*29*) and in the isomerase reaction (Eq. 10). As shown in Fig. 1, $f^{5\text{-}10}FH_4$ or $f^{10}FH_4$ is the formylating agent in all other reactions.

Interconversion of Formyl and Hydroxymethyl Groups with Methyl Groups

Although the details of the enzymatic pathways concerned with thymine and methionine synthesis are not fully known, the methyl groups of these compounds have been shown to be interconvertible with formyl and hydroxymethyl groups (*5*). It seems probable also that the transfer of the C_1 group occurs at the hydroxymethyl level rather than at the formyl level (*38*). In thymidine synthesis, uracil deoxyriboside (*39*) [or ribotide (*40*)], rather than free uracil, has been implicated as the C_1 acceptor. In methionine synthesis, homocysteine serves as the acceptor. The above reactions may be generalized in the following sequence, where *A* represents either uracil deoxyriboside or homocysteine, *h-A* the corresponding hydroxymethyl derivatives, and *m-A* the methyl derivatives:

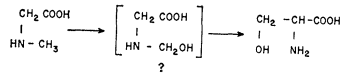
$$hFH_4 + A = h\text{-}A + FH_4 \quad (15a)$$

$$h\text{-}A \underset{(2H)}{\rightleftharpoons} m\text{-}A \quad (15b)$$

Both thymidine (*39, 40*) and methionine (*41*) syntheses have been studied in crude extracts, and it has been shown that the required components are (i) the acceptor (uracil deoxyriboside or homocysteine); (ii) a hydroxymethyl source and FH_4; (iii) a reducing source, usually DPN, although this may not be the actual [2H] shown in reaction 15b; and (iv) ATP, perhaps to promote the condensation in reaction 15a.

Reaction 15b is of great interest when uracil deoxyriboside is the acceptor because it seems most probable that the over-all requirement for vitamin B_{12} in thymine synthesis can be narrowed down to this step. The possibilities that the porphyrin-bound cobalt of vitamin B_{12} passes through an oxidoreduction cycle during reaction 15b deserve investigation.

The sarcosine-to-serine conversion (*42*) is also of interest, for it represents the hydroxymethyl → methyl sequence in reverse and is carried out intramolecularly:



(16)

This reaction has been demonstrated in intact mitochondria (*42*) or digitonin extracts of mitochondria (*43*), but the cofactor requirements or intermediates are presently unknown.

Biosynthesis of Folic Acid Coenzymes

The above reactions emphasize the role of reduced folic acid (FH_4) as the coenzyme, or carrier, of the C_1 groups. The formation of FH_4 from the vitamin, folic acid, has been the subject of recent investigations. Wright *et al.* (*44*) have shown in a bacterial system that folic acid is reduced to dihydrofolic acid during operation of the pyruvic oxidase. In chicken liver extracts both steps in the reduction of folic to tetrahydrofolic acid require TPNH (*45, 46*).

In our laboratory (*47*), a TPN-linked, dihydrofolic reductase has been purified from avian liver and shown to catalyze the final step in FH_4 biosynthesis:

$$FH_2 + TPNH + H^+ \rightarrow FH_4 + TPN^+ \quad (17)$$

The equilibrium at *p*H 7 for reaction 17 lies far to the right. At extremely low concentrations (about $10^{-7}M$), the folic acid antagonists, aminopterin and amethopterin, are extremely potent competitive inhibitors of FH_2 in the reaction (*48*).

Summary

The above reactions involving "active formyl" and "active hydroxymethyl" groups, their interconversion, their connections with free HCOOH and HCHO, and their interdigitation with methyl groups are diagrammed in Fig. 1.

References and Notes

1. R. J. Winzler, A. D. Williams, W. R. Best, *Cancer Research* 17, 108 (1957).
2. F. Bethell and M. Swenseid, *J. Clin. Invest.* 25, 917 (1946).
3. M. Swenseid, F. Bethell, O. D. Bird, *Cancer Research* 11, 864 (1951).

4. E. L. R. Stokstad, in *The Vitamins*, W. H. Sebrell, Jr., and R. S. Harris, Eds. (Academic Press, New York, 1954), vol. 3, pp. 124–142.
5. W. Sakami, in *Amino Acid Metabolism*, W. D. McElroy and H. B. Glass, Eds. (Johns Hopkins Press, Baltimore, Md., 1955), pp. 658–683.
6. We are indebted to our colleagues Dr. P. T. Talbert, Dr. Y. Hatefi, Dr. E. J. Ordal, and Mrs. L. D. Kay for many stimulating discussions of these problems. The experimental work cited herein was made possible by research grants from the Life Insurance Medical Research Fund, the U.S. Public Health Service, the Atomic Energy Commission [contract No. AT(45-1)-783], and the institutional grant to the University of Washington made by the American Cancer Society.
7. For an extensive review of this field, see G. R. Greenberg and L. Jaenicke in *Chemistry and Biology of Purines*, G. E. W. Wolstenholme and C. M. O'Connor, Eds. (Little, Brown, Boston, 1957), pp. 204–232.
8. F. M. Huennekens, Y. Hatefi, L. D. Kay, *J. Biol. Chem.* **224**, 435 (1957).
9. S. Deodhar and W. Sakami, *Federation Proc.* 12, 195 (1953).
10. R. L. Blakley, *Biochem. J.* **58**, 448 (1954); R. L. Kisliuk and W. Sakami, *J. Biol. Chem.* **214**, 47 (1955).
11. N. Alexander and D. M. Greenberg, *J. Biol. Chem.* **214**, 821 (1955).
12. L. Jaenicke, *Biochim. et Biophys. Acta* 17, 588 (1955).
13. E. Vercamer *et al.*, unpublished results.
14. The following abbreviations have been used: F, folic acid; FH_2, 7,8-dihydrofolic acid; FH_4, 5,6,7,8-tetrahydrofolic acid; fFH_4 and hFH_4, formyl and hydroxymethyl tetrahydrofolic acid (position of the one-carbon unit not specified); f^5FH_4 and $f^{10}FH_4$, N^5- and N^{10}-formyl tetrahydrofolic acid; $f^{5\text{-}10}FH_4$, N^5,N^{10}-methenyl tetrahydrofolic acid; h^5FH_4, $h^{10}FH_4$, and $h^{5\text{-}10}FH_4$, the corresponding hydroxymethyl derivatives of FH_4; fi^5FH_4, N^5-formimino FH_4; FIG, formiminoglycine; FIGlu, formiminoglutamic acid; DPN and DPNH, TPN and TPNH, oxidized and reduced di- and triphosphopyridine nucleotide; ADP, AMP, and ATP, adenosine di-, mono-, and triphosphate; PP, pyrophosphate; Pi, inorganic phosphate; CoA, coenzyme A.
15. B. E. Wright, *Biochim. et Biophys. Acta* **16**, 165 (1955).
16. D. E. Metzler, L. B. Longenecker, E. E. Snell, *J. Am. Chem. Soc.* 75, 2787 (1953); 76, 639 (1954).
17. M. J. Osborn *et al.*, *ibid.* 79, 6565 (1957).
18. R. L. Blakley, *Biochem. J.* 65, 331 (1957).
19. Because the term *active hydroxymethyl* is already established in the literature, it should be retained even though a structure like $h^{5\text{-}10}FH_4$ represents a methylene, rather than a hydroxymethyl, derivative of FH_4.
20. L. Jaenicke, *Federation Proc.* 15, 281 (1956).
21. G. R. Greenberg, L. Jaenicke, M. Silverman, *Biochim. et Biophys. Acta* 17, 589 (1955).
22. Y. Hatefi *et al.*, *J. Biol. Chem.* 227, 637 (1957).
23. J. C. Rabinowitz and W. E. Pricer, Jr., *J. Am. Chem. Soc.* 78, 5702 (1956).
24. R. L. Kisliuk, *J. Biol. Chem.* 227, 805 (1957).
25. M. J. Osborn *et al.*, *Biochim. et Biophys. Acta* 26, 208 (1957).
26. G. R. Greenberg, *Federation Proc.* 13, 745 (1954).
27. H. R. Whiteley, M. J. Osborn, F. M. Huennekens, *J. Am. Chem. Soc.* 80, 757 (1958).
28. R. A. Smith, I. F. Frank, I. C. Gunsalus, *Federation Proc.* 16, 251 (1957).
29. M. Silverman *et al.*, *J. Biol. Chem.* 226, 83 (1957).
30. G. R. Greenberg, *J. Am. Chem. Soc.* 76, 1458 (1954).
31. J. M. Peters and D. M. Greenberg, *J. Biol. Chem.* 226, 329 (1957).
32. L. D. Kay *et al.*, unpublished observations.
33. M. P. Schulman, J. C. Sonne, J. M. Buchanan, *J. Biol. Chem.* 196, 499 (1952); L. Warren, *Federation Proc.* 16, 267 (1957).
34. J. C. Rabinowitz and W. E. Pricer, Jr., *J. Am. Chem. Soc.* 78, 4176 (1956); *J. Biol. Chem.* 222, 537 (1956).
35. A. Miller and H. Waelsch, *Arch. Biochem. Biophys.* 63, 263 (1956); H. Tabor and J. C. Rabinowitz, *J. Am. Chem. Soc.* 78, 5705 (1956).
36. C. H. Chin, L. O. Krampitz, G. D. Novelli, *Bacteriol. Proc. Soc. Am. Bacteriologists* 57, 127 (1957).
37. H. R. Whiteley, unpublished results.
38. D. Elwyn, A. Weissbach, D. B. Sprinson, *J. Am. Chem. Soc.* 73, 5509 (1951); *J. Biol. Chem.* 207, 467 (1954).
39. R. L. Blakley, *Biochim. et Biophys. Acta* 24, 224 (1957); E. A. Phear and D. M. Greenberg, *J. Am. Chem. Soc.* 79, 3737 (1957).
40. M. Friedkin and A. Kornberg, in *Chemical Basis of Heredity*, W. D. McElroy and H. B. Glass, Eds. (Johns Hopkins Press, Baltimore, Md., 1957), pp. 609–614.
41. A. Nakao and D. M. Greenberg, *J. Am. Chem. Soc.* 77, 6715 (1955).
42. C. G. Mackenzie and R. H. Abeles, *J. Biol. Chem.* 222, 145 (1956).
43. R. C. Knowles and W. Sakami, Abstr. 36C, 132nd Meeting Am. Chem. Soc., New York, 1957.
44. B. E. Wright, M. L. Anderson, E. C. Herman, *J. Biol. Chem.* 230, 271 (1958).
45. S. Futterman, *ibid.* 228, 1031 (1957).
46. S. F. Zakrzewski and C. A. Nichol, *Biochim. et Biophys. Acta* 27, 425 (1958).
47. M. J. Osborn and F. M. Huennekens, unpublished results.
48. M. J. Osborn, M. Freeman, F. M. Huennekens, *Proc. Soc. Exptl. Biol. Med.* 97, 429 (1958).

Optical Tracking of Artificial Earth Satellites

The Moonwatch program and the precision photographic program supply much of the raw orbit data.

Fred L. Whipple

Mankind's curiosity in exploring the unknown and his ability to use the accumulated experience and knowledge of the race have resulted in his creation of nine astronomical bodies in the course of the past seven months. Five artificial satellites have been put into orbit about the earth, and three of these involved additional pieces of equipment that have constituted independent satellites. The fact that the life-times of these various artificial satellites are relatively short compared to the life-times of natural satellites does not detract from their astronomical significance or from the intellectual and technological achievement that they represent. All satellites, whether natural or artificial, are temporary in nature, if one chooses a sufficiently long time scale. Man-made satellites have now become so numerous that the people most intimately connected with the programs are beginning to have difficulty in segregating and recalling the individual characteristics of each one. Hence, for the convenience of both the writer and the reader, G. F. Schilling has kindly prepared Table 1, listing the major basic information concerning the identification, nature, initial orbit, and survival characteristics of these various satellites. Identification is by year, in the order of launching according to the Greek alphabet. When more than one component of a launching is optically detectable, the components are identified by Arabic numerals following the Greek letter, in order of decreasing optical brightness. Other entries of Table 1 are self-explanatory or are described in the legend.

The orbital elements of artificial satellites are subject to fairly rapid and major changes arising from the earth's deviation from sphericity and from the effect of atmospheric drag. The major effect of the earth's equatorial bulge is to produce a regression of the orbital nodes—the intersection of the equatorial and orbital planes—in a westerly direction for eastward-moving satellites such as now are aloft. The rate depends upon the orbital inclination and physical dimensions. The direction from the center of the earth to minimum distance (peri-

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