ELECTRONIC STRUCTURE AND BIOCHEMICAL FUNCTION OF FOLIC ACID COENZYMES*

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SUMMARY

The two principal biochemical functions of folic acid coenzymes—acting as reversible carriers of one-carbon metabolic groups or as electron donors—are interpreted in terms of the electronic structure of these compounds. The acceptance of the one-carbon unit by N_5 of tetrahydrofolic acid is attributed to the relatively high electronic charge and free valence of that atom. The utility of the isomerisation of the inactive form of the coenzyme, which results from this first reaction, into the active form, in which the one-carbon unit is fixed either at N_{10} or shared by N_5 and N_{10}, may reside in the fact that the departure of the one-carbon unit must be easier in these latter isomers. The electron deficiency on the bond to be ruptured, as measured by its dipositivity is effectively greater when the one-carbon unit is fixed on N_{10} or shared between N_5 and N_{10} than when it is fixed on N_5.

The study of the energies of the molecular orbitals leads to the conclusion that tetrahydrofolic acid must be a good electron donor. The study of the electronic structure of folic acid, leads to the prediction, which seems to be in agreement with experiment, that its reduction should be easier at the 7–8 bond than at the 5–6 bond. The possible reduction product N_5–N_8 dihydrofolic acid presents the unusual property of having an antibonding highest filled orbital and should consequently resist further reduction since it is a particularly good electron donor.

INTRODUCTION

Folic acid (I), a vitamin of the B group, is converted *in vivo* through a series of enzymic transformations into a coenzyme form, which is an essential reversible carrier of a one-carbon metabolic group, at the oxidation level of formate or formaldehyde[1]. The class of enzymes which utilise the folic acid coenzymes are known under the general name of "pteroproteins".

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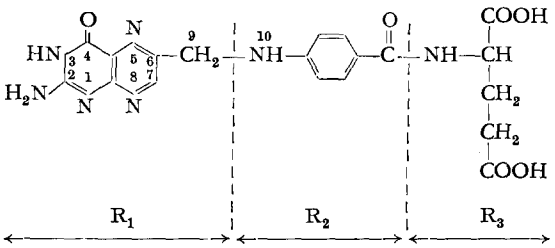
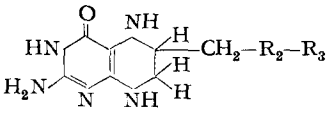


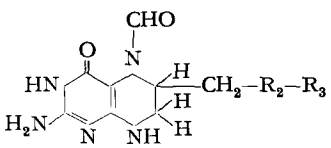
I. Folic acid (F)



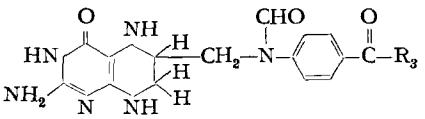
II. Tetrahydrofolic acid (FH_4)

The coenzyme form of folic acid (symbol F) is the 5,6,7,8-tetrahydrofolic acid (II) (symbol FH_4). The general pattern of its biological function as a carrier of one-carbon unit, *e.g.* the formyl unit (symbol f), may be resolved into three stages:[2-4]

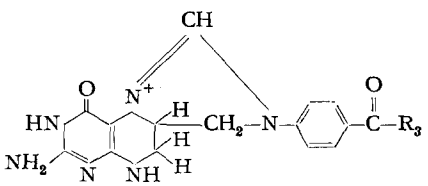
(1) The acceptance of the one-carbon group from a donor. The one-carbon unit becomes attached to N_5 of the coenzyme giving rise to e.g. 5-formyl-FH_4 (III) or folinic acid (symbol f_5FH_4)*.



III. f_5FH_4



IV. $f_{10}FH_4$



V. $f_{5-10}FH_4$

(2) This form of the coenzyme, which is inactive[5,6], is then transformed enzymically into an active form which may be either 10-formyl-FH_4 (IV) (symbol $f_{10}FH_4$) or the bridge compound N_5,N_{10}-methenyl FH_4 (V) (symbol $f_{5-10}FH_4$).

(3) Following the specificity of the enzyme either $f_{5-10}FH_4$ or $f_{10}FH_4$[7-9] give up their formyl group to an appropriate receptor, regenerating FH_4.

Among the most important reactions catalyzed in this way by the folic acid coenzymes are some essential steps in the *de novo* synthesis of the purines of the nucleic acids, especially the incorporation of C_2 and C_8 into the skeletons of these bases[10-13].

A second important biochemical function of the folic acid coenzymes is to act as electron donors, particularly in the formation of the methyl groups of choline and thymine from formaldehyde[14-16]. The reaction necessitates the oxidation of FH_4 to FH_2.

In connection with this aspect of the biochemical role of folic acid, there is also

* In ATP requiring enzymic reactions, the formyl group is transferred directly to position 10 of the 10-phosphorylated FH_4. These reactions will be discussed in a subsequent paper.

the problem of the structure and significance of the dihydrofolic acids (symbol FH_2) which represent the possible intermediate steps in the reduction of F to FH_4 or the reoxidation of FH_4 to F. The information available in this field is complex and does not lead to any straightforward conclusion. Thus, *e.g.* FUTTERMAN[17] has purified an enzyme system which catalyzes both the reduction of F to FH_2 (TPNH dependent reaction) and the reduction of FH_2 to FH_4 (DPNH or TPNH dependent reaction), a result which suggests a two steps biosynthesis of FH_4 with an intermediate formation of an FH_2. On the other hand WRIGHT *et al.*[18–20] who have studied the enzymic reduction of F by folic reductase purified from *C. Sticklandii* according to the reaction

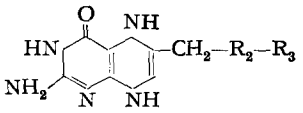
$$F + CoASH + \text{pyruvate} \longrightarrow FH_2 + CoASCOCH_3 + CO_2$$

(in which pyruvate is the electron donor) have not been able to reduce further the FH_2 which is formed to FH_4; the F–FH_2 couple functions as an oxido-reduction system, F being the electron acceptor and FH_2 the electron donor. Nevertheless, GREENBERG *et al.*[15,21] showed the existence in mammalian tissue (rat thymus) of a reversible enzymic reduction of FH_2 to FH_4

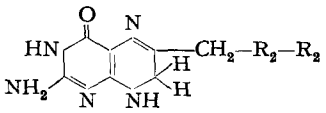
$$FH_4 + DPN^+ \rightleftarrows FH_2 + DPNH$$

A similar reaction, in avian and mammalian livers, mediated by a TPN-linked dehydrogenase, has been observed by OSBORN AND HUENNEKENS[22].

The question occurs then, of course, as to the structure (or the structures) of the dihydrofolic acid (or acids) which are formed as intermediates in these different transformations of F to FH_4 or *vice-versa*. PETERS AND GREENBERG[14] suggest as the active forms of FH_2, obtained by enzymic oxidation of FH_4, either N_5–N_8 FH_2 (VI) or C_7–N_8 FH_2 (VII). OSBORN AND HUENNEKENS[22], more exclusive, estimate that the enzymic reoxidation of FH_4 yields C_7–N_8 FH_2. BLAKLEY[23,24] who observed that FH_2, obtained by enzymic or chemical reduction of F, yields the 5-hydroxymethyl derivative through the action of HCHO, deduces from this result that the reduction obviously involves position 5; but the results do not permit it to be ascertained whether this takes place at positions 5–8 or 5–6[25]. The question is thus unsettled.



VI. N_5–N_8 FH_2



VII. C_7–N_8 FH_2

The present investigation was undertaken with the aim of contributing towards the elucidation of the mechanism of action of the folic acid coenzymes by establishing in particular the factors responsible for their functioning as carriers of one-carbon units and for their involvement in hydrogen transfer reactions. It also aimed towards the elucidation of the structure and properties of the different possible dihydro intermediates between F and FH_4. Finally, a further aim of this investigation was the possible application of the results to the study of the mechanism of action of folic acid antimetabolites, in particular of antimetabolites which, like aminopterin or amethopterin, are active in cancer chemotherapy[26–29]. Their activity seems to be related to the blocking of the enzymic reduction of F to FH_4, and it may consequently be

hoped that the elucidation of the reactions involved in these transformations may throw light on the mechanism of action of the antimetabolites. However, this problem of antimetabolites will be treated in a separate publication.

METHODS

The method employed for the study of the electronic structure of the compounds involved is the L.C.A.O. approximation of the molecular orbital method of quantum chemistry[30], with our usual appropriate parameters[31]. The only new case was that of the quaternary nitrogen of $f_{5-10}FH_4$. Previously, we have used for the Coulomb integral of the quaternary nitrogen of DPN^+ the value $\alpha_{N^+} = \alpha_C + 2\beta$[32]. As the positive charge in $f_{5-10}FH_4$ resonates between two nitrogens, N_5 and N_{10}, the previous value of α_{N^+} seemed to be too great for the present case and, consequently, a reduced value of $\alpha_N = \alpha_C + 1.5\beta$ has been assumed for both the N_5 and N_{10} of $f_{5-10}FH_4$.

Folic acid, its derivatives and coenzymes may, in principle, manifest keto–enol tautomerism. Since a study of hydroxypteridines has shown that these molecules exist essentially in the keto form[33], we have considered only this form for all the compounds discussed in this paper.

The structure of folic acid is obviously complex and is composed of three fragments: the pteridine nucleus (R_1), the *para*aminobenzoic system (R_2) and the glutamic acid residue (R_3). Of this last fragment, only the N–H group has been introduced into the calculations. The remaining portion of the residue is separated from R_1 and R_2 by a saturated carbon and consequently does not participate in the general electronic conjugation.

Folic acid has consequently been considered as a system composed of a 2,amino-4,hydroxypteridine, united through the hyperconjugation of a CH_2 group to a *para*-aminobenzoylamine. It is thus a unique system of 28 π electrons.

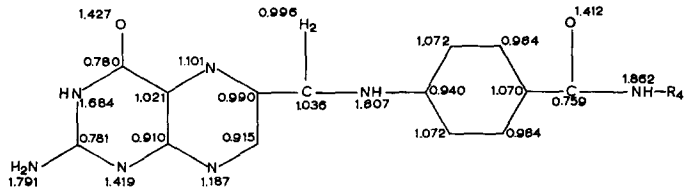
FH_4 on the contrary, because of the saturation of C_6, has to be considered as composed of two separated units, which are the hydrogenated pteridine nucleus (14 π electrons) and the *para*aminobenzoic acid fragment (12 π electrons). No interaction of mobile electrons exist, at a first approximation, between these two portions of the coenzyme. The situation is similar in the formylated derivatives f_5FH_4 and $f_{10}FH_4$. It is only in the bridge compound $f_{5-10}FH_4$ that direct π electron interaction is established between the two portions of the molecule, through the methenyl bridge. This last molecule is thus a unique system of 26 mobile electrons[34].

RESULTS AND DISCUSSION

Folic acid coenzymes as one-carbon unit carriers

As one-carbon unit acceptors: Following the excellent definition proposed by KOSHLAND[35], group transfers may be considered as enzymic substitution reactions. Consequently one may plausibly seek to interpret them in terms of structural characteristics succesfully employed for the interpretation of substitution reactions in general. These characteristics may be looked for in the first place in the electronic properties of the atoms constituing the conjugated substrate which undergoes the substitution. There are two principal properties to be considered: the electronic charges and the free valences of the atoms[30,36].

Fig. 1 represents the distribution of the electronic charges in folic acid and in tetrahydrofolic acid. Limiting ourselves to the problem considered in this section and examining the charge distribution in tetrahydrofolic acid, which is the one-carbon unit acceptor, we may obviously draw the following first conclusion: the one carbon unit is being accepted by the hydrogen carrying N atom of FH_4 which has the greatest charge of the mobile or π electrons.



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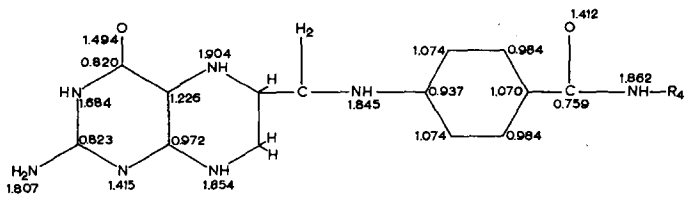


Tetrahydrofolic acid (II)

Fig. 1. Electronic charges (see text).

As is well known, when an NH group is incorporated into a conjugated system, the "lone pair" of electrons situated on this N atom effectively participates in the conjugation (the delocalisation) of the π electrons of the system. The result is a discharge of those nitrogen atoms, a fraction of the electrons of the lone pair migrating into the conjugated ring. Because of this electronic delocalisation these N atoms keep less than two electrons: they carry, consequently, a certain formal positive charge which represents the difference between the 2 electrons which the N atom would possess in the absence of any conjugation and that of its effective electronic charge[37]. Thus *e.g.* the formal positive charge of the N_5 of FH_4 is $2e - 1.904e = 0.096e$ and it may be observed that it is the smallest of the formal positive charges carried by the NH groups in FH_4. It is thus the N_5 of the FH_4 skeleton which out of all similar atoms conserves the greatest fraction of its lone pair and it is on this atom that the one-carbon group is fixed.

As a matter of fact the N_5 of FH_4 has also another outstanding property. Thus Fig. 2 represents the values of the free valences of all the hydrogen-carrying nitrogens of FH_4 (these free valences being obtained by following the usual procedure of substracting from a constant, taken in this case to be equal to 1.42, the sum of the mobile

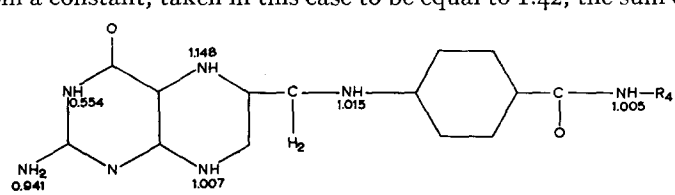
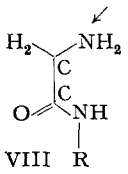


Fig. 2. Free valences (see text).

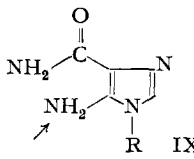
bond orders of the bonds adjacent to each N) and it may be observed that it is N_5 again which also has the greatest free valence. Consequently, it may also be said that the one carbon unit is accepted by the NH group of FH_4 whose nitrogen has the greatest free valence.

The significance of this second correlation may be two-fold. Thus, in the first place, the free valence of an atom is a direct measure of its ability to undergo substitution reactions with radicals so that a large free valence means in general a high affinity for free radicals. In the second place the magnitude of the free valence gives also, generally, a good indication as to the polarisability of an atom by approaching reagents, whatever they are. A large free valence means high polarisability. Consequently, the large free valence of the N_5 of FH_4 signifies a low activation energy for the substitution of a radical on this atom and, considered together with the relatively high electronic charge of that atom, signifies also a low activation energy for the electrophilic substitution of that atom. The formylation reaction of FH_4 at N_5 may thus use one or both of these mechanisms.

The study of the electronic structure of the principal biological receivers of the formyl group transferred by the folic acid coenzymes leads to a consideration of a similar mechanism of formylation in their case also. Among such principal receivers are glycinamide ribotide (VIII) and 5-amino, 4-imidazole carboxamide ribotide (IX), two important intermediates in the *de novo* synthesis of nucleic acids[10,38,39]. The formylation of these two compounds occurs at the nitrogens indicated by arrows on the formulas.



VIII R



R = Ribose-phosphate

R IX

The distribution of electrical charges in VIII and IX is indicated in Fig. 3 and the free valences of the nitrogens susceptible *a priori* to undergo formylation are given in Fig. 4[40]. It may be observed that in VIII the nitrogen which receives the formyl group has both the highest electronic charge and the largest free valence. In IX, the nitrogen which receives the formyl group also has the highest free valence, but has a smaller electronic charge than the other available nitrogens. It would thus seem plausible that the free valence may perhaps be a better index than the electronic charge for measuring the aptitude of hydrogen carrying nitrogens to undergo formylation.

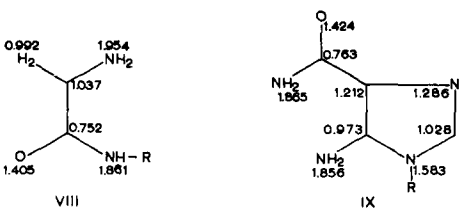


Fig. 3. Electrical charges (see text).

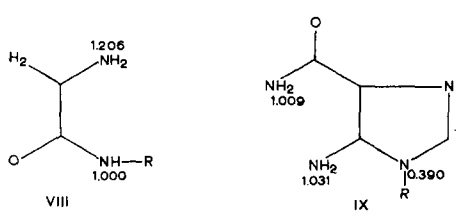


Fig. 4. Free valences (see text).

Finally the results concerning the formylation of FH_4 and related compounds may be compared to those concerned with the action of formaldehyde on FH_4 and

on some other fundamental biochemicals[1]. Thus FH_4 serves as a coenzyme for the enzymic system which synthetizes serine from glycine and formaldehyde. The first step in this synthesis consists in a non-enzymic combination of formaldehyde with FH_4[41]. According to BLAKLEY[41,42] the reaction leads probably to the formation of N_5–N_{10}-methylenetetrahydrofolic acid, through the intermediate formation of N_5-hydroxymethyltetrahydrofolic acid. The reaction of formaldehyde with the N_5 of FH_4 is thus probably the first step in the reaction and the electronic factors responsible for it are certainly the same as those considered for enzymic formylation.

The role of these factors may also be noticed in the action of formaldehyde on the purine and pyrimidine bases of the nucleic acids. According to FRAENKEL-CONRAT[43] formaldehyde interacts with the three bases which possess an NH_2 group, adenine, guanine and cytosine, with the probable formation of a Schiff base ($-N=CH_2$). The relative reactivities as judged from the approximate increase in the u.v. absorption are in the order: adenylic acid > cytidylic acid > guanylic acid.

This may be compared with the electronic characteristics of the nitrogens of the amino group of the bases[44] (Table I).

TABLE I

PROPERTIES OF THE NH_2 GROUPS

Compounds	*Electrical charge on N of NH_2*	*Free valence of N of NH_2*
Adenine	1.310	0.960
Cytosine	1.803	0.944
Guanine	1.803	0.933

We observe that both the electronic charge and the free valence of the N atom of NH_2 are larger in adenine than in the remaining two bases. The great relative reactivity of adenine towards formaldehyde may consequently, following the general ideas developed in this paper, be attributed to this state of affairs. On the other hand, the nitrogens of the amino groups of cytosine and guanine have identical electrical charges. Consequently, it is the value of the free valence of these atoms which must be responsible for their relative reactivities and this free valence is greater in cytosine than in guanine. Effectively, cytosine is more reactive than guanine. This result could again point to the importance of free valence in determining the reactivity of heterocyclic nitrogens towards one carbon units at the oxidation stage of formate or formaldehyde. It is striking to observe that the theoretical considerations developped here are able to account for the rather surprising order of reactivity towards formaldehyde which places a pyrimidine ribotide between two purine ribotides.

Folic acid coenzymes as one-carbon unit donors: f_5FH_4 does not function as a formyl donor. In order to become a formyl donor, this inactive form of the coenzyme must be transformed into $f_{10}FH_4$ or $f_{5-10}FH_4$ which are the active forms. It is again in the electronic characteristics of these different forms that we shall seek the explanation for the differences in their ability to give up their extracyclic one-carbon unit.

Fig. 5 represents the distribution of the electrical charges in f_5FH_4, $f_{10}FH_4$ and $f_{5-10}FH_4$. The examination of those distributions of charges, in relation with the problem considered in this section, leads to the following principal conclusion: the ability of the coenzyme to give up the one-carbon unit may be related to the di-

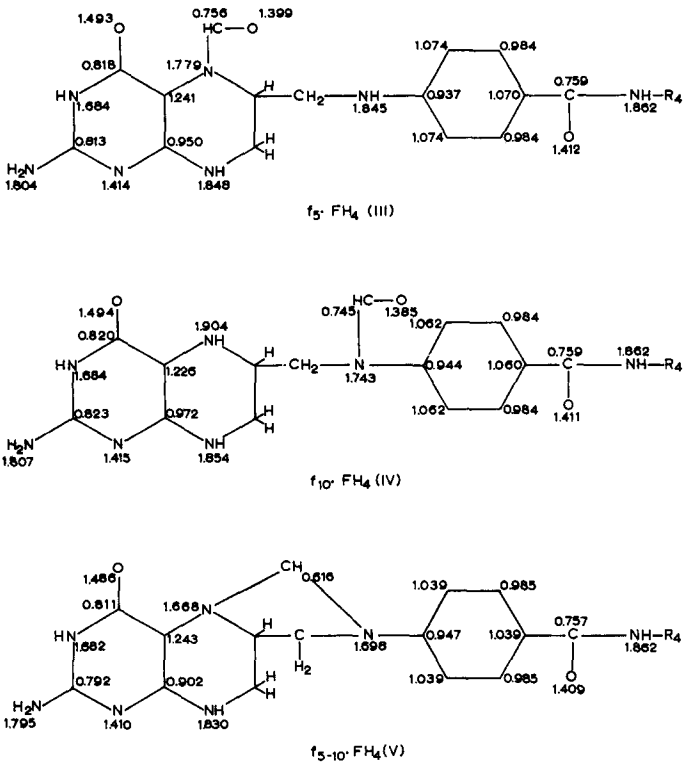


Fig. 5. Electrical charges (see text).

positivity of the N–C bond which has to be ruptured. This dipositivity is greater in the active forms of the coenzyme than in the inactive one.

The term "dipositivity" may need an explanation[37]. It can be observed that both the nitrogen atom of FH_4 upon which the formyl group is fixed and the carbon atom of this group are electron deficient. They both carry formal positive charges which may be calculated by subtracting from 2 the electronic charge on the nitrogen atom and from 1 the electronic charge on the carbon atom. The "dipositivity" of the bond refers to the presence of such formal positive charges on both of the atoms of the bond. In $f_{5-10}FH_4$ there are, naturally, two such bonds around the one-carbon unit. Table II contains an evaluation of this dipositivity of the bond to be ruptured for the different forms of the formylated coenzyme.

TABLE II

DIPOSITIVITY OF BONDS

Compound	*Formal positive charge on the N atom carrying the formyl group*	*Formal positive charge on the C atom of the formyl group*	*Dipositivity of the N–C bond*
f_5FH_4	0.221	0.244	0.465
$f_{10}FH_4$	0.257	0.255	0.512
$f_{5-10}FH_4$	$N_5 = 0.332$ $N_{10} = 0.302$	0.384	0.716 0.686

As stated before this dipositivity is appreciably greater in the active forms than in the inactive one and it seems, consequently, plausible to admit that it is the value of this dipositivity which is responsible, at least to some extent, for the relative ability of the different forms to give up the one-carbon unit. A larger dipositivity represents greater electronic repulsion in the bond under consideration, so that the larger its electronic deficiency the weaker the bond. This conclusion is substantiated by similar results obtained recently in the study of the electronic structure of the energy-rich phosphates[45] and the electronic structure of the principal types of biochemical bonds undergoing enzymic hydrolysis[37]. In both cases the dipositivity of the ruptured bonds was an essential feature of their structure. Thus the isomerisation of the inactive f_5FH_4 into the active $f_{10}FH_4$ or $f_{5-10}FH_4$ leads to a more economically functioning form of the coenzyme. As a confirmation of this point of view it may be useful to quote the results of the determination of the free energies of the (non-enzymic) hydrolysis of the compounds cited. The relative free energies of hydrolysis at pH 7 stand in the order $f_{5-10}FH_4 > f_{10}FH_4 > f_5FH_4$[1] a result which points out at the same time the greater lability of the one-carbon unit in $f_{5-10}FH_4$ or $f_{10}FH_4$ than in f_5FH_4 and the important role of the degree of electron deficiency of a bond for the ease of its hydrolysis. Another striking observation in this field is that, in purified extracts of *Clostridium cylindrosporum*, $f_{5-10}FH_4$ is opened selectively to $f_{10}FH_4$ by the enzyme cyclohydrolase. The bond which is hydrolysed is the most electron deficient of the two susceptible ones, a result which is again in agreement with the elements of the general theory of enzymic hydrolysis as proposed by B. AND A. PULLMAN.

In this field, again, it may be useful to investigate whether the one-carbon unit donor properties of other important biochemical substances may also be related to the same factor. That this seems to be essentially the case may be concluded from the important observation that while formiminoglutamic acid, a degradation product of histidine[2,46–48], is an important formylation agent for the enzymic synthesis of the active coenzyme, formylglutamic acid is unable, in the same conditions, to give up its one-carbon unit. The distribution of electrical charges in these two substances is given in Fig. 6 and the dipositivity of the susceptible bonds evaluated in Table III.

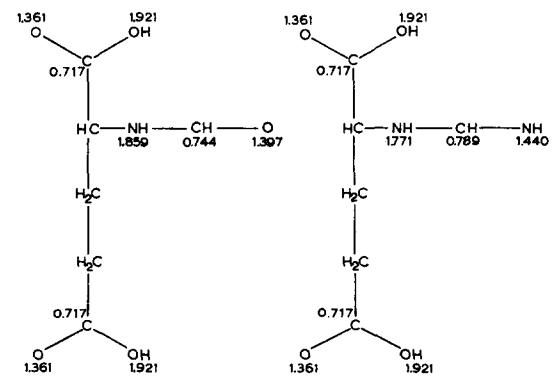


Fig. 6. Electronic charges (see text).

It can be seen that the bond maintaining the one-carbon unit is more dipositive in the active formiminoglutamic acid than in the inactive formylglutamic acid.

TABLE III

DIPOSITIVITY OF BONDS

Compound	*Formal positive charge on the N atom carrying the formyl group*	*Formal positive charge on the C atom of the formyl group*	*Dipositivity of the N–C bond*
Formylglutamic acid	0.141	0.256	0.397
Formiminoglutamic acid	0.229	0.211	0.440

Finally, one may consider, as a complementary factor which favors $f_{10}FH_4$ as the active form of the one-carbon unit donor, the variation of the resonance energy of the coenzyme which accompanies the departure of the formyl group. The departure is, as far as the folic acid coenzyme is concerned, accompanied by a decrease in its resonance energy. This decrease is nevertheless smaller for the departure of the formyl group from the N_{10} of FH_4 than from its N_5, the corresponding resonance energy variations being, respectively, 0.371 β and 0.400 β.

Electron (hydrogen) donor and acceptor properties of di- and tetrahydrofolic acids

As shown recently by B. AND A. PULLMAN the electron donor and acceptor properties of biochemicals may be satisfactorily accounted for in terms of the energies of their highest filled and lowest empty molecular orbitals[49]. The same considerations will now be applied to the theoretical investigation of these properties in the di- and tetrahydrofolic acids, in connection with the different experimental observations in this field quoted briefly in the introducting remarks of this paper.

Table IV contains the energies of the highest occupied molecular orbital (h.o.m.o.) and the lowest empty molecular orbital (l.e.m.o.) of F and of the pteridine fraction of FH_4 and of the two isomeric FH_2's: the N_5–N_8FH_2 and the C_7–N_8FH_2. In the hydrogenated derivatives of F, it is this pteridine portion of the molecule which is effectively the essential center of the electron transfer properties and, as it is to a large extent isolated from the remaining parts of the compound, it is the only one which needs to be considered here. (For information it may be quoted that the *para*aminobenzamide central fraction of these compounds should have negligible electron transfer capacities, the coefficients of its h.o.m.o. and l.e.m.o. being respectively +0.555 and −1.)

TABLE IV

ENERGIES OF MOLECULAR ORBITALS

Compound	*Energy of the highest filled molecular orbital*	*Energy of the lowest empty molecular orbital*
F	+0.446	−0.648
The pteridine fraction of FH_4	+0.048	−1.071
The pteridine fraction of N_5–N_8 FH_2	−0.237	−0.927
The pteridine fraction of C_7–N_8 FH_2	+0.293	−0.748

The results of Table IV indicate that:

(a) Folic acid itself should be a moderate electron donor or acceptor, according to the conditions. Its moderate electron acceptor properties have been demonstrated recently by FUJIMORI who showed that this compound was able to form to some

extent charge transfer complexes with tryptophan (acting as an electron donor)[50]. Nevertheless this ability was much smaller for folic acid than, *e.g.*, for riboflavin.

(b) The distribution of the energy levels in FH_4 is profoundly different from that in the parent F. FH_4 appears to be an excellent electron donor, a property confirmed by its ready reoxidation to dihydrofolic acid, folic acid and degradation products[42]. Its electron acceptor properties are bound to disappear, a conclusion again confirmed by its inability to form a charge transfer complex with tryptophan[50].

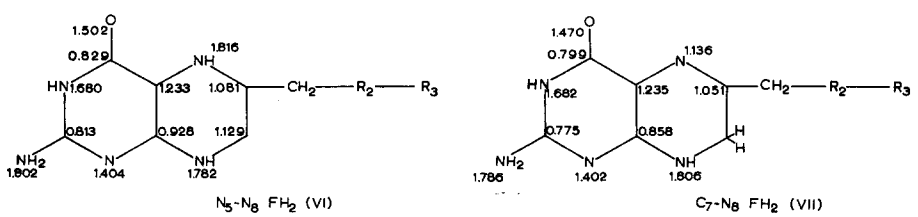
(c) The two dihydrofolic acids considered should be electron donors, but in this respect the electron donor properties of N_5–N_8FH_2 should be particularly outstanding. In fact, this molecule manifests the unusual property, first discovered by B. AND A. PULLMAN for the case of the reduced form of the riboflavin coenzymes, of possessing an antibonding highest occupied orbital[49]. Such a property must be associated with an usually low ionization potential and extremely pronounced electron donor capacities.

In view of these results it seems tempting to postulate that it is the formation of this N_5–N_8FH_2 which occurs in the course of the reduction of F at conditions at which it does not appear to be possible to continue the reduction till the formation of FH_4. The unusually strong electron donor properties of this compound preclude such a continuation and lead to a reversible dehydrogenation.

Correlatively, the formation of the C_7–N_8 FH_2 may be postulated as an intermediate in the reductions which end in FH_4.

We have omitted from our considerations the third possible isomer N_5–C_6FH_2 both because the experimental evidence seems to preclude its formation and because such a preclusion is justified on theoretical grounds. Effectively, the examination of the appropriate electronic index of folic acid leads to the conclusion that of the two reductions—the saturation of either the N_5–C_6 bond or that of the C_7–N_8 bond—the second one is far more probable. Thus, as the two reductions represent addition reactions to a bond, and most probably atomic addition reactions, the relative ease of the reduction at these two similar bonds will be determined essentially by (a) the value of the mobile order of the bond and by (b) the free valences[30] of the reactive atoms, in the present case especially, of the N_5 and N_8 atoms. The bond orders of the N_5–C_6 and C_7–N_8 bonds of F are, respectively, 0.656 and 0.683 and the free valences of N_5 and N_8 0.120 and 0.158[34]. The reduction of folic acid at the C_7–N_8 bond appears, consequently, to be more probable than its reduction at the N_5–C_6 bond. Moreover, this reduction also leads to a more stable isomer, the resonance energies of the pteridine residues of the two dihydrofolic acids being, respectively, 3.197 β for C_7–N_8FH_2 and 3.041 β for the N_5–C_6 isomer. It may be useful to add that the resonance energy for the N_5–N_8FH_2 is 3.525 β.

We reproduce in Fig. 7 the distribution of electrical charges in the two probable FH_2 forms.



N_5-N_8 FH_2 (VI) C_7-N_8 FH_2 (VII)

Fig. 7. Electrical charge (see text).

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