BIOORGANIC CHEMISTRY 3, 229–237 (1974)

On Corrin Biogenesis

A. IAN SCOTT, EUN LEE, AND CRAIG A. TOWNSEND

Sterling Chemistry Laboratory, Yale University, New Haven, Connecticut 06520

Received October 23, 1973

In the light of recent experimental evidence a theory of corrin biogenesis is proposed which involves a symmetry-allowed concerted electrocyclic reaction as the key step.

The early and, by now, classic experiments of Shemin (*1*), in which the origin of the modified pyrrole rings of the ligand system of vitamin B_{12} was defined as δ-aminolevulinic acid (ALA), (formed in turn from glycine and succinate), set the stage for speculation on the relationship between the iron (heme), magnesium (chlorophyll), and cobalt (corrin) pathways in Nature. With the knowledge that porphobilinogen (PBG) (*2*) also serves as a precursor for these three important classes of natural product, several hypotheses have been adduced for the mechanism of corrin biosynthesis (*3*, *4*) with emphasis on the point at which the three structural types diverge in their genesis.

Thus, it was proposed by Corwin and Mathewson (*5*) that both type III porphyrinogen and corrins may have a common precursor in the form of a linear tetrapyrrole which can cyclise to give either uroporphyrinogen (uro'gen) III or a corrin-like macrocycle. Another suggestion (*6*) has implicated uroporphyrin III (as the cobalt complex) and a cyclopropane intermediate which eventually generates the methyl group at C_1.

The latter scheme and several others involving cyclopropane chemistry have now been rendered untenable by the finding that, although a small amount of radioactivity finds its way from C_5 of ALA to the C_1 and other methyl groups by secondary processes (*1*), the results of feeding both [5-^{13}C]-ALA and [$^{13}CH_3$]methionine leave no doubt that the methyl group at C_1 (α) as well as those at $C_2(\alpha)$, C_5, $C_7(\alpha)$, $C_{12}(\alpha)$, C_{15}, and $C_{17}(\beta)$ originate from methionine, decarboxylation of an acetic acid residue of a PBG unit providing the β-methyl group at C_{12} (*7–9*). A summary of these recent feeding experiments is shown in Fig. 1.

The Corwin mechanism (and the several other remaining theories) could also be discounted if uro'gen III were shown to be an obligatory intermediate. Our recent work with ^{14}C- and ^{13}C-enriched uro'gens (*10*) indicates that uro'gen III (but not uroporphyrin III) is indeed a biointermediate (Fig. 2), regardless of the mechanism of uro'gen III formation from 4 moles of PBG (*11*, *12*). The complete and rigorous experimental proof for the intermediacy of the intact uro'gen III molecule must await the results of feeding a regiospecifically doubly labeled sample of pure uro'gen III, but recent cell-free work (*13*) supports the premise that the corrin chromophore is biosynthesised by the introduction of seven methionine-derived methyl groups in to the uro'gen III molecule.

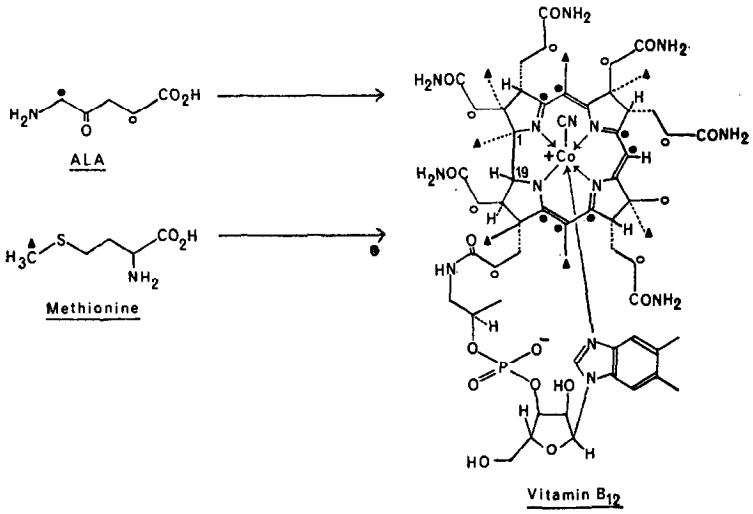


FIGURE 1

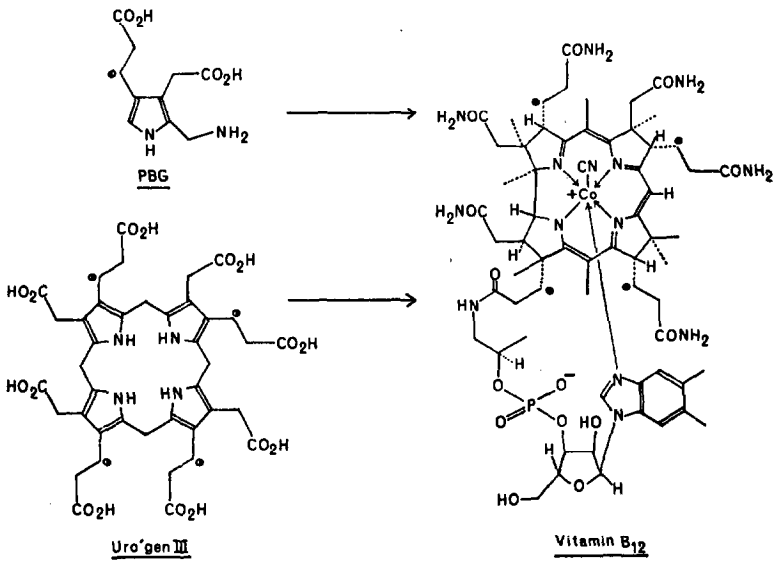


FIGURE 2

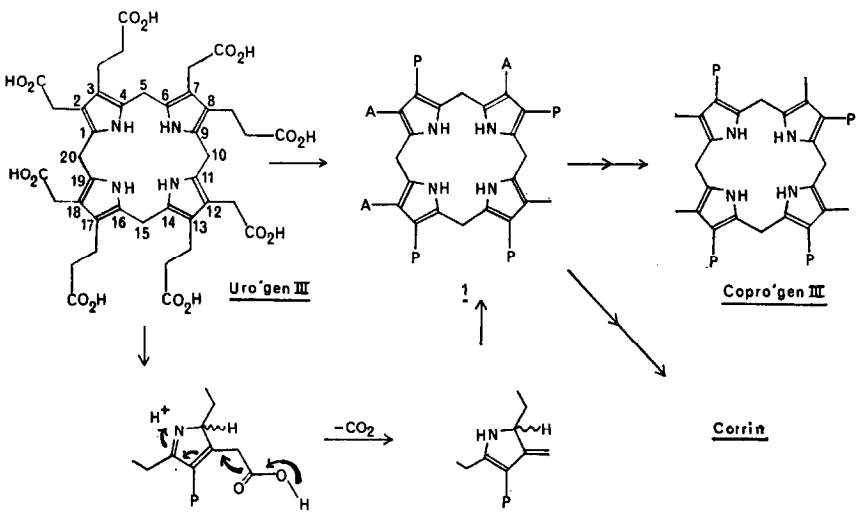
In accord with all of the published experimental evidence the following requirements must be met in the conversion of uro'gen III to cobyrinic acid.

1. Decarboxylation of the acetic acid side chain at C_{12}.
2. Loss of the *meso* carbon at C_{20} and formation of a new bond between C_1 and C_{19}.
3. Introduction of the seven "extra" methyl groups from *S*-adenosylmethionine (SAM).
4. Reduction (four electron equivalents).
5. Insertion of cobalt.

These requirements impose considerable limitations on any mechanistic proposals and at the same time render untenable many of the previous suggestions regarding corrin biogenesis. In order to guide the design of future experiments in this area and to fill the void left by the relinquishment of earlier ideas on corrin biogenesis, we have developed the following hypothetical sequence which may provide several interesting possibilities open to experimental test.

1. Decarboxylation

With the demonstration of a cell-free system capable of transforming uro'gen III to cobyrinic acid (*13*) we propose that the first event in this sequence be the decarboxylation of the acetic acid side chain at C_{12}. Since, in all biological decarboxylations described hitherto, the presence of an electron sink is obligatory, we suggest that decarboxylation should occur prior to methylation at C_{12}. The enzymatic decarboxylation of uro'gens to coproporphyrinogens (copro'gens) by the ubiquitous uro'gen decarboxylase is a well-known process (*14*, *15*), intermediates having been isolated in the partially decarboxylated form. Although the exact structures of the heptacarboxylic porphyrins have not been defined, the low substrate specificity of these decarboxylases implies that there is probably formed an equal amount of each possible geometric isomer, one of which will be implicated in the biosynthesis of vitamin B_{12} (Scheme 1).

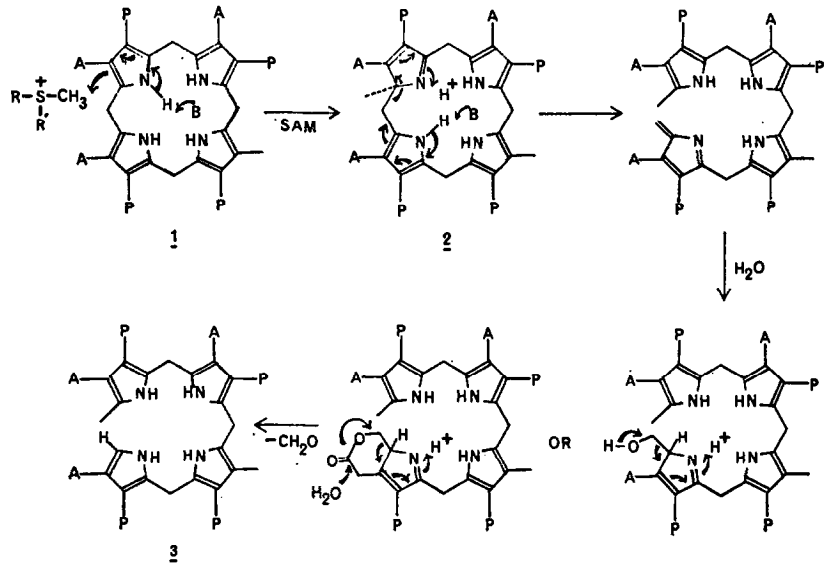


SCHEME 1

2. Methylation and Loss of C_{20}

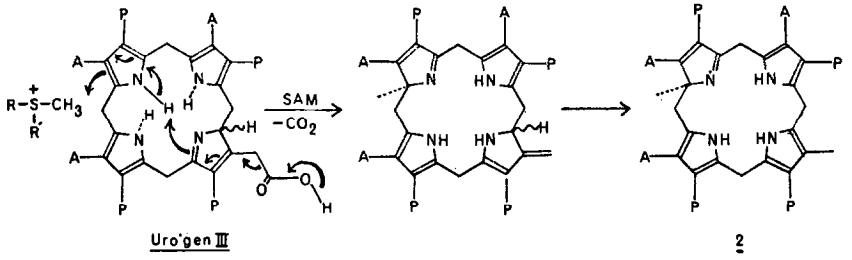
The heptacarboxylic porphyrinogen **1** can undergo the first methylation at C_1 by SAM, presumably coupled with a base-catalyzed hydrogen abstraction. The methylated species **2** is now poised for a rearrangement process in which formaldehyde is lost via hydration, generating the linear tetrapyrrole **3**. We note that this step is equivalent to two-electron reduction (Scheme 2).

It is also instructive to consider that these first steps, viz. decarboxylation and methylation, could occur simultaneously in favorable conditions (Scheme 3). By operation of



SCHEME 2

such a push–pull mechanism, it is possible to reduce further the arbitrary nature of the scheme, which would then require a specific decarboxylase/methyl-transferase.

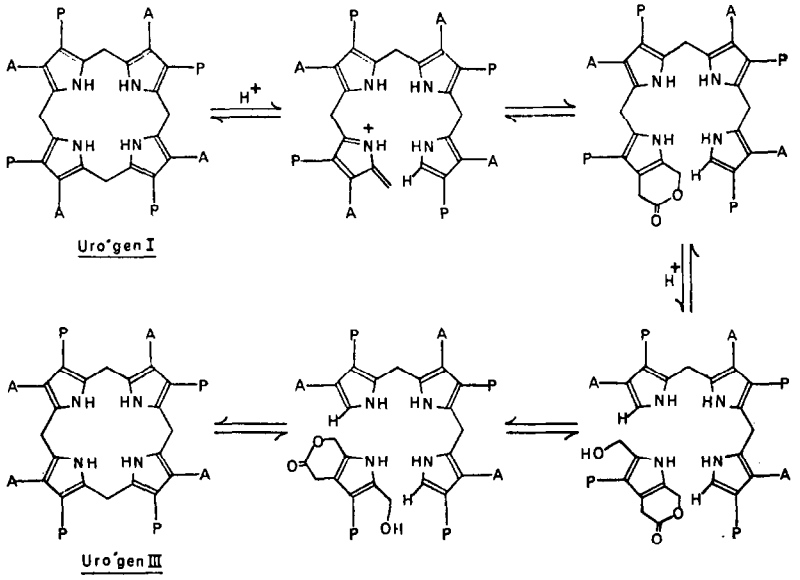


SCHEME 3

To explain the acidic *in vitro* isomerization of uro'gens, Mauzerall has proposed (*16*) ring cleavage coupled with protonation as a working hypothesis as illustrated in Scheme 4. At the same time, the latter author established that the yield of uro'gen dropped markedly when the reaction was performed in the presence of dimedone, an efficient formaldehyde-trapping agent, whereas exogenous formaldehyde equilibrated freely with the *meso* carbons. Here, we find a close analogy to Scheme 2, viz. methylation, ring cleavage, and loss of formaldehyde.

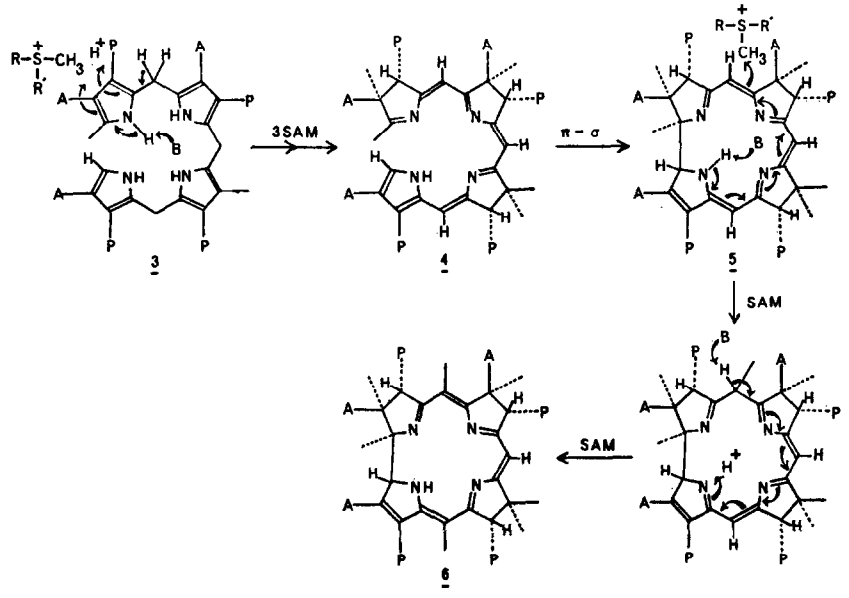
3. *Further Methylations and Cyclization*

Subsequent consecutive methylations at C_2, C_7 (see note in proof), and C_{12}, in which the conformation of the substrate and the direction of approach of the incoming methyl groups are apparently controlled by the methylating enzyme, provide a conjugated



SCHEME 4

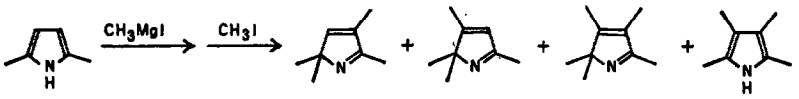
16π-electron array **4**, which may now undergo an orbital-symmetry-allowed π-σ rearrangement in a concerted manner to produce a dehydrocorrin chromophore **5** upon which C_5 and C_{15} methylations may operate (26) (Scheme 5).



SCHEME 5

Electrophilic substitution usually favors the α-position rather than the β-position of pyrroles (*17*, *18*), but the preference is not overwhelming and certainly much less in pyrroles than in furans and thiophenes. For instance, low, but comparable yields of

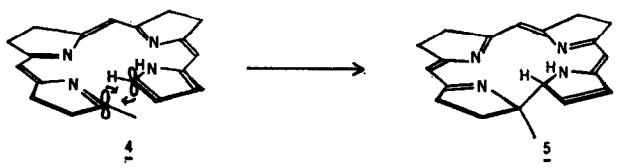
both α- and β-methylated products were obtained from 2,5-dimethylpyrrolylmagnesium iodide with methyl iodide (*19*) (Scheme 6).



SCHEME 6

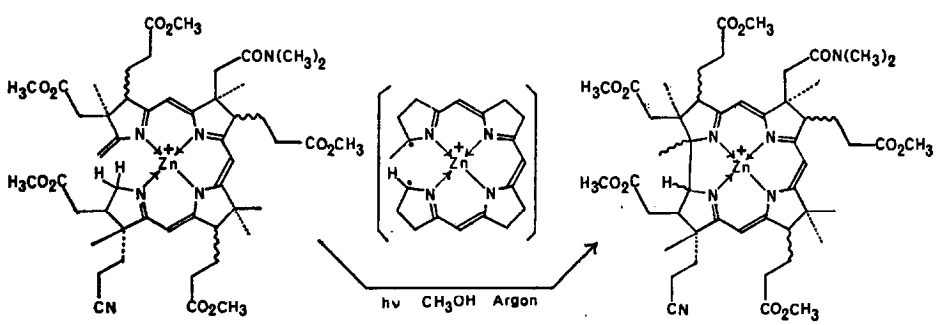
In the case of substrate **3**, the energy stabilization resulting from an extensive delocalization of π-electrons should facilitate β-methylations on pyrrole units. An interesting outcome of the above scheme is the possibility of C_{12} methylation from the α-face in unison with C_2 and C_7 methylations, in accord with the observed chirality of the methylation process (*8*) (see note added in proof).

Close examination of the model structures reveals that the electrocyclic reaction calls for a minimum alteration in atomic arrangements, i.e., the delocalized π-orbital has an ideal Möbious overlap for a conrotatory ring closure to achieve the observed *trans* stereochemistry of the C_1 methyl and C_{19} hydrogen (Scheme 7).



SCHEME 7

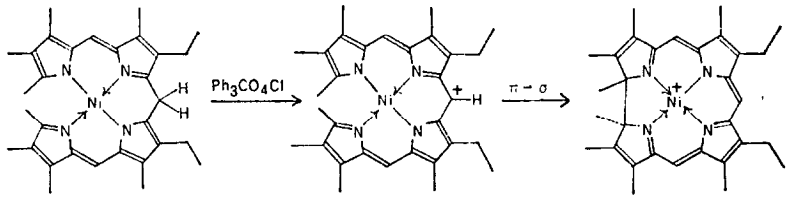
Such a process is reminiscent of and isoelectronic with the second (thermally allowed) part of Eschenmoser's remarkable photochemical synthesis of corrins (*20*) (Scheme 8)



SCHEME 8

and also finds excellent analogy in the elegant cyclization studies of tetradehydrocorrins by Johnson (*21*) (Scheme 9).

Both authors cite the template effect of the central metal atoms in their substrates which ensure close proximity of the interacting centers and prevent severe distortions of the π-electron system. Although the possible involvement of cobalt or any other metal



SCHEME 9

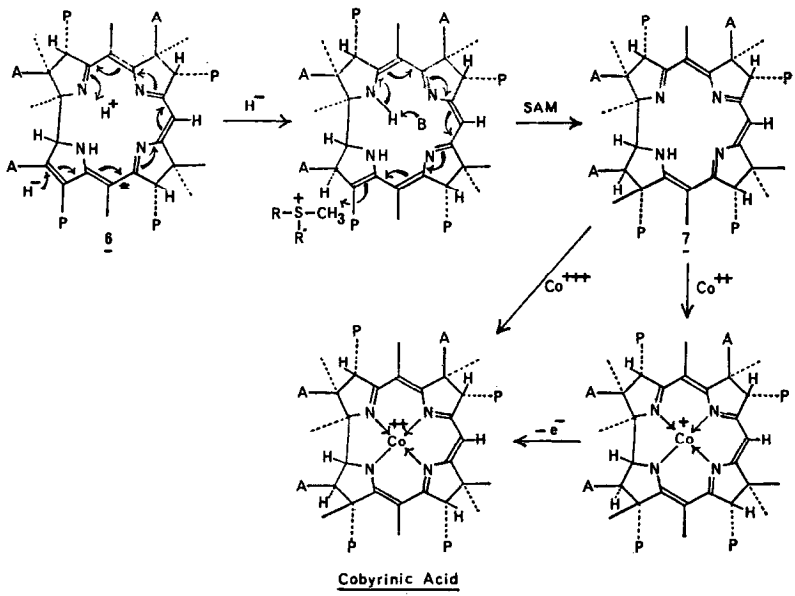
ion in this process cannot be ruled out, it seems reasonable to assume that the conformation of an intermediate may depend totally on enzyme specificity without the aid of a template metal atom.

4. Reduction and Methylation

The resultant 18-dehydro-17-desmethyl cobalt-free cobyrinic acid **6** is now aligned for a final reductive methylation sequence. Following the conjugative attack of hydride (possibly from NADPH) at C_{18} a study of models suggests that α-side of the nascent corrin is more sterically encumbered than the β-face and the attack of SAM should occur from the less-hindered β-face at C_{17} which is now activated toward electrophilic substitution (Scheme 10). The product, cobalt-free cobyrinic acid **7** may well be a precursor of the family of naturally occurring cobalt-free corrins (*22*, *23*).

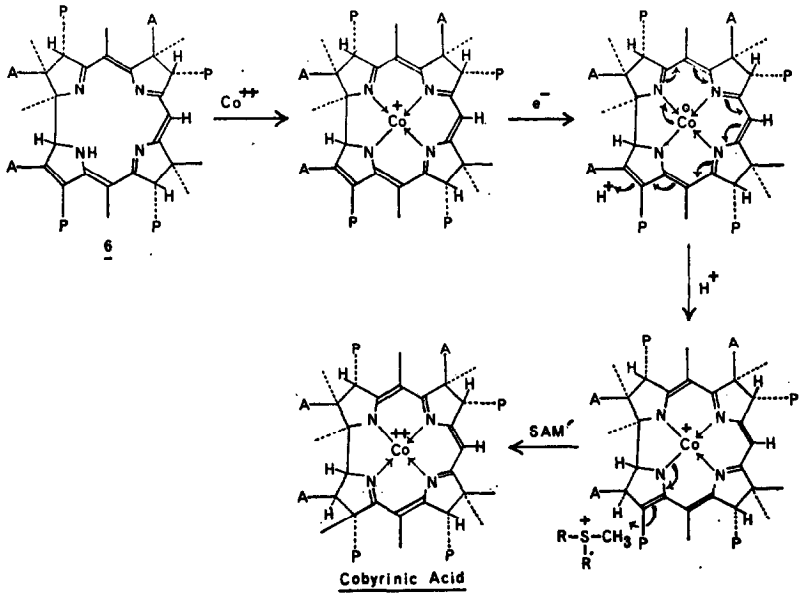
5. Insertion of Cobalt

The incorporation of cobalt atom poses the last problem in the sequence. The cobaltous ion (Co^{2+}) may be oxidized before or after incorporation into the ring (Scheme 10).



SCHEME 10

On the other hand, the possible utilization of the extra electron in Co^{2+} for C_{17} methylation cannot be discounted. For instance, the addition of Co^{2+}, a methyl group and a proton with supply of one electron to the precorrin system **6** will produce cobyrinic acid (Scheme 11). One-electron reductions are well known in vitamin B_{12} enzymology in conjunction with the biological formation of coenzyme B_{12} (*24*).



SCHEME 11

The further metabolism of cobyrinic acid to the cobalamins and coenzyme B_{12} has been exhaustively studied by Bernhauer in *P. shermanii* (*25*) and the full sequence of successive amidation and addition of the aminoisopropanol and nucleotide segments has been determined.

In conclusion we feel that the above hypothesis offers a rationale for the uro'gen → corrin transformation which can be tested at several crucial points where the stability of certain of the putative intermediates would allow inhibition and/or feeding studies to be conducted.

Notes added in proof. β-methylation at C_2 and C_7 provides a rationall for an alternative structure for Sirohydrochlorin (L. M. Siegel, M. J. Murphy, and H. Kamin. *J. Biol. Chem.*, **248**, 251 (1973).

A conflicting result concerning the stereochemistry at C_{12} has been disclosed (C. E. Brown, D. Shemin, and J. J. Katz, *J. Biol. Chem.*, **248**, 8015 (1973).

ACKNOWLEDGMENT

We thank the National Institutes of Health (Grant AM17014-01) for support and Dr. E. Gordon for stimulating discussions.

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