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Preface

The enzymes of heme biosynthesis (fig. 1) continue to attract the attention of investigators in a wide variety of disciplines. However, research progress in this field has been impeded by the lack of convenient and reliable assays for these enzymes whose substrates and products are often unstable or difficult to quantitate. During the past several years, new and more sensitive methods have been developed for each enzyme of the heme biosynthetic pathway. Therefore, our goal was to compile into one volume, specific, sensitive, and reliable assays for each of these enzymes. It was our hope that this 'handbook' of assay methods would be a useful resource for seasoned investigators as well as a convenient guide for the expeditious assay of these enzymes by those new to this field.

To this end, experienced investigators were invited to contribute 'state-of-the-art' assays for each of the eight enzymes in the heme biosynthetic pathway (fig. 1). Emphasis was to be placed on the accurate determination of activities in both crude and purified preparations using the approach of the enzymologist. Contributors were requested to indicate modifications or precautions necessary for different species, various tissue sources or clinical applications. In addition, 'tricks' and pitfalls peculiar to a given procedure were to be included.

A notable feature of this volume is the fact that each method was independently evaluated by another laboratory having expertise with the specified enzyme. These investigators were requested to reproduce the assay, confirm that similar results could be independently obtained and contribute additional helpful comments. These 'confirmations' appear immediately following each assay procedure.

For several enzymes, more than one assay method is presented, reflecting different approaches or experimental constraints. For example, the colorimetric method for ALA synthase is reliable for induced tissues and other sources which have high activity. In contrast, the more sensitive radiochemical assay is preferred for most normal tissues in which ALA synthase activity is quite low. The fluorometric assay is also valuable for sources with low activities but has the advantage of being the most rapid method. Since the scope of this volume was limited to the heme biosynthetic pathway, assays for porphobilinogen oxygenase, heme oxygenase and other catabolic enzymes were omitted. Assays for dioxovalerate transaminase and plant ALA synthase, the alternate pathways for ALA synthesis, also were not included.

This compilation of assays presented the opportunity to compare the relative activities of all the heme biosynthetic enzymes in a single tissue source. Each contributor was asked to determine a specific activity in rat liver to serve as a benchmark for comparison and for

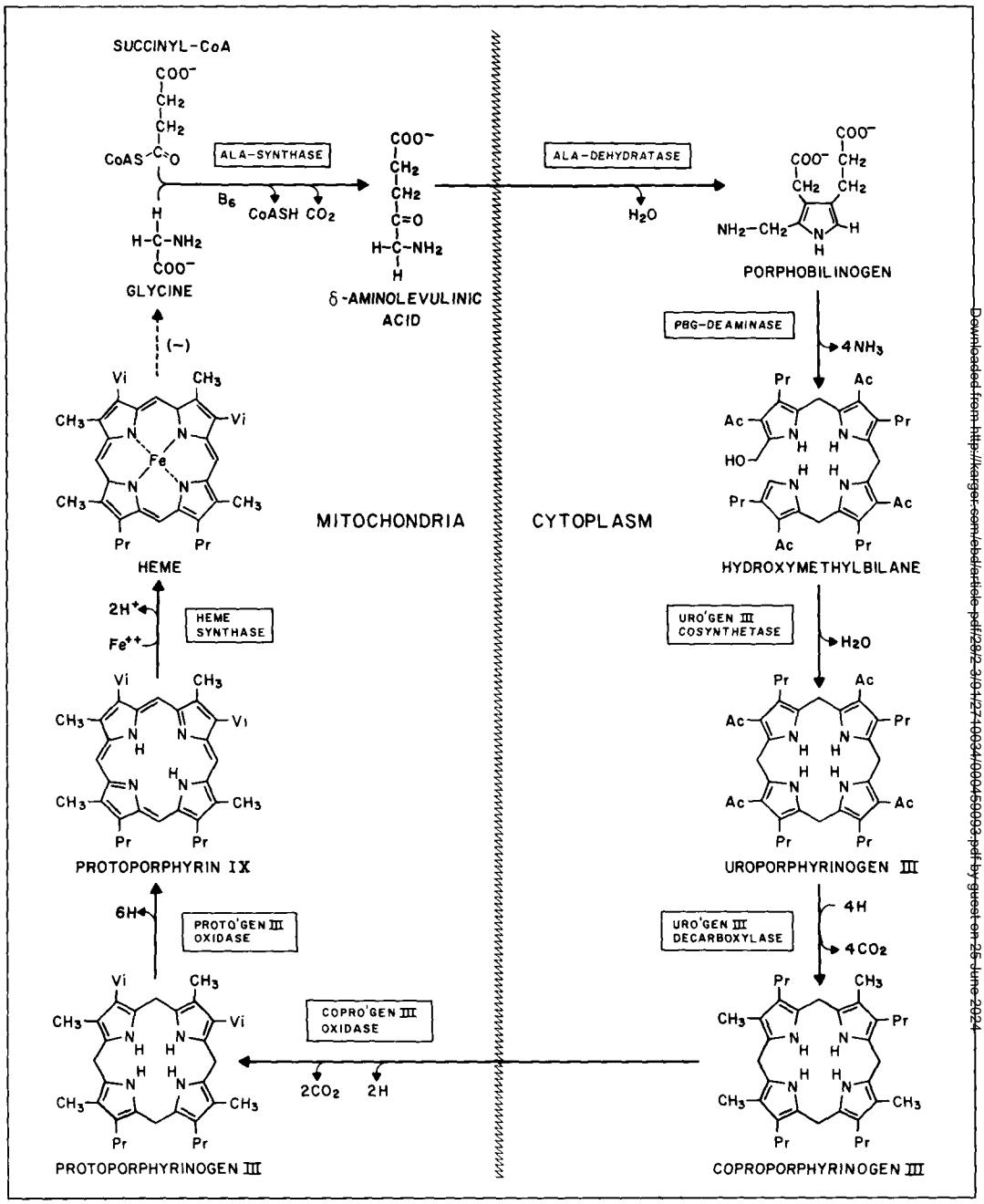

Fig. 1. Current concept of heme biosynthesis. B6 = Pyridoxal-5'-phosphate; ALA = δ-aminolevulinic acid; PBG = porphobilinogen; URO'GEN = uroporphyrinogen; COPRO'GEN = coproporphyrinogen; PROT'GEN = protoporphyrinogen; Ac = acetate; Pr = propionate; Vi = vinyl.

Table I. Activities of the heme biosynthetic enzymes in adult rat liver

Enzyme	Enzyme activity[1] U/g	Reference[2]	Mean activity U/g	Relative activity[3]
ALA synthase	8.7	*Bishop* et al.	41	1.0
	14.0	*Bottomley and Moore*[4]		
	11.9	*Brooker* et al.[5]		
	5.6	*De Matteis*		
	105	*Lien and Beattie*[6]		
	102	*Pomeroy and Bonkowsky*[6]		
ALA dehydratase	2,220	*Sassa*	1,650	80
	1,540	*Jordan and Gibbs*		
	1,190	*Giampietro and Desnick*		
PBG deaminase	12.6	*Anderson and Desnick*	13.7	2.7
	14.7	*Bishop and McBride*		
URO III cosynthetase	520	*Jordan*	770	150
	1,020	*Straka* et al.		
URO decarboxylase	282	*Straka* et al.[5]	318	62
	291	*Felsher and Carpio*		
	319	*Elder and Wyvill*		
	380	*de Verneuil and Nordmann*		
COPRO oxidase	140	*Grandchamp and Nordmann*	147	29
	153	*Elder and Smith*		
PROTO oxidase	150	*Jacobs and Jacobs*[5]	150	29
HEME synthase	2,320	*Bloomer and Morton*	2,320	450

[1] One unit (U) of activity is that amount of enzyme required to form 1 nanomole of product per hour at 37 °C. The activities in rat liver homogenates or extracts are expressed per gram wet liver weight.

[2] Authors of the assay methods and confirmations in this issue (from where this data was taken).

[3] To obtain the relative in vitro heme biosynthetic activity, each activity was converted to nanomoles of ALA equivalents produced per hour per gram liver and then was divided by the mean ALA synthase activity.

[4] Data corrected to activity at 37 °C.

[5] Personal communications; values based on calculations from data reported in this issue.

[6] It is not clear why this colorimetric method gave higher ALA synthase activities. It is notable that the fold increase of hepatic ALA synthase following allylisopropylacetamide induction was similar to that found by other colorimetric assays which gave lower activities for uninduced liver (*Bonkowsky*, et al., this issue, p 130).

assay confirmation. Table I summarizes these results. ALA synthase was clearly the rate-limiting enzyme in the pathway, followed by PBG deaminase which had a threefold higher specific activity. Of course, these in vitro levels represent only an approximation of the in vivo activities.

It has been a pleasure collaborating with the contributors to this volume. Their enthusiastic response and cooperation were especially appreciated. The 'confirmation authors' deserve special commendation for their willingness to set up and reproduce assays designed by others.

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The Editors