Studies on the Efflux of Metalloporphyrin from Rat-Liver Mitochondria

EFFECT OF ALBUMIN, GLOBIN, HAEMIN AND HAEMOGLOBIN

Paul HUSBY and Inge ROMSLO

Laboratory of Clinical Biochemistry, University of Bergen, N-5016 Haukeland Sykehus, Bergen, Norway

(Received 26 October 1979)

The mechanism by which metalloporphyrins escape from mitochondria has been studied in isolated rat-liver mitochondria using Co-deuteroporphyrin as the model compound. During the first 10–15 min of incubation the efflux is about 10% of the total amount of Co-deuteroporphyrin synthesized. The efflux then increases to a second steady-state level of 25–35% after 30–45 min of incubation. The efflux is inversely correlated to the energy state of the mitochondria. Globin at concentrations $>0.4\mu\text{mol/l}$ enhances the efflux of Co-deuteroporphyrin, but has no effect on the degree of energy coupling or on the rate of Co-deuteroporphyrin synthesis. The effect of globin can be competitively inhibited by adding haemin. Haemin ($0.5\text{--}1.0\mu\text{mol/l}$) when added to the medium in the absence of globin reduces the efflux of Co-deuteroporphyrin by 20–30%, but has no effect on the metal-chelatase activity. Neither albumin nor haemoglobin increases the efflux of Co-deuteroporphyrin from intact mitochondria. The results suggest that the efflux of metalloporphyrin is regulated in part by the energy state of the mitochondria and in part by the presence of metalloporphyrin-binding ligands and unattached haemin in the incubation medium.

The final reaction in the biosynthesis of haem is catalysed by ferrochelatase (EC 4.99.1.1), an enzyme that is reported to be firmly bound to the matrix side of the mitochondrial inner membrane (Jones & Jones, 1969). Haem thus produced is utilized in part outside the mitochondria (Marver *et al.*, 1966; Garner & McLean, 1969; Marver, 1969; Higashi *et al.*, 1974) and in part within the mitochondria (Korb & Neupert, 1978). The mechanisms by which haem reaches the cytosol is not known (Yoda & Israels, 1972; Israels *et al.*, 1975), neither is it known whether haem binds to its apo-protein on the endoplasmic reticulum (Davidian & Penniall, 1971), in the cytosol (Felicetti *et al.*, 1966), at the inner mitochondrial membrane (Ponka *et al.*, 1973; Korb & Neupert, 1978) or within the mitochondria (Schiefer, 1969).

Ponka *et al.* (1973) found that albumin and globin enhanced the efflux of [^{59}Fe]haem from rabbit reticulocytes. Essentially similar results were reported by Israels *et al.* (1975) from studies on the efflux of haem from rat-liver mitochondria. In a sucrose medium, only 10% of the amount of haem synthesized leaked to the medium during a 30 min

incubation. In the presence of albumin, a marked increase in this efflux was observed. Also, cytosol markedly increased this efflux. The results from these experiments are difficult to interpret in terms of transport, because no data were given as to the morphological and functional integrity of the mitochondria (U. Müller-Eberhard, in 'Discussion' of Israels *et al.*, 1975).

Other compounds of the cytosol that may control the intracellular movement of haem are haemopexin (Müller-Eberhard & Morgan, 1975; Morgan, 1976), ligandin (Ketterer *et al.*, 1976), ferrihaemoglobin (Schulman, 1974) and Z-protein (Tipping *et al.*, 1976).

In previous papers we reported on the uptake of iron (Romslo & Flatmark, 1973a) and porphyrins (Koller & Romslo, 1978) by isolated mitochondria. We now report on the efflux of Co-deuteroporphyrin from isolated rat-liver mitochondria.

Materials and Methods

Materials

Rat-liver mitochondria were prepared as described (Romslo & Flatmark, 1973b). The functional integrity of the preparations was tested by measuring the respiratory control ratio with ADP,

Abbreviation used: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethane sulphonic acid.

using succinate as the substrate. Only mitochondria with this ratio >4 at the start of the experiments were used.

The structural integrity of the mitochondria was determined from the leakage of malate dehydrogenase into the incubation medium. Less than 1% of the malate dehydrogenase activity of the mitochondria leaked into the incubation medium during a 60 min incubation.

Synthesis and efflux of Co-deuteroporphyrin

Mitochondria, about 2 mg of protein/ml, were preincubated at 30°C for 1 min in a medium containing in a volume of 26 ml: 0.25 M-sucrose, 5 mM-Hepes buffer, pH 7.40, and 2.0 μ M-deuteroporphyrin. Further additions were as described in the legends to the Figures. The reaction was initiated by adding 8 μ M- $^{57}\text{CoCl}_2$ (30 000 c.p.m./nmol).

At timed intervals aliquots (1 ml) were withdrawn and transferred to 3 ml of ice-cold cyclohexanone containing 60 μ l of 8 M-HCl. The suspension was agitated on a vortex mixer for 30 s, left on ice for 30 min and the phases were separated by centrifugation at 2000 g for 5 min. The supernatant organic phase was removed and the radioactivity of an aliquot was determined in an LKB-Wallac 80000 gamma sample counter to a precision of $\pm 2\%$. All determinations were done in duplicate.

To determine the efflux of Co-deuteroporphyrin a similar aliquot was taken from the incubation medium, centrifuged at 13 000 g for 2 min at 4°C in an Eppendorf microcentrifuge (type 3200) and the supernatant was treated with cyclohexanone containing HCl as described above. The malate dehydrogenase activity of the supernatant in the zero-time incubation was less than 1% of the total, i.e. the mitochondria were not disrupted during the centrifugation procedure.

The extraction of Co-deuteroporphyrin into cyclohexanone was markedly influenced by pH. From a protein-free medium the extraction of Co-deuteroporphyrin was essentially complete at pH <0.5 . The extraction was also markedly influenced by the protein concentration of the incubation medium. With increasing amounts of protein (albumin, globin or mitochondria) the extraction of Co-deuteroporphyrin decreased to reach a level of about 60% at 1.5–2 mg of protein/ml. Therefore, to correct for differences in extraction of Co-deuteroporphyrin into cyclohexanone, parallel experiments were carried out in which ^{57}Co -deuteroporphyrin taken up in cyclohexanone was re-extracted into incubation mixtures containing appropriate amounts of protein. The loss of radioactivity (%) from the cyclohexanone phase thus determined was used to correct the radioactivities of the corresponding supernatants and mitochondrial samples. On average, the extraction from the protein-poor supernatants was

90–95% and from the whole mitochondrial samples about 60%.

Other analytical procedures

Mitochondrial respiration rates were determined by a Clark oxygen electrode (Biological Oxygen Monitor; Yellow Springs Instrument Co., Yellow Springs, OH, U.S.A.) with appropriate polarization circuitry. The mitochondria, about 2 mg of protein, were incubated at 30°C in a medium containing in a final volume of 3 ml: 50 mM-glucose, 175 mM-sucrose, 10 mM-Hepes, pH 7.40, 5 mM-MgCl₂ and 5 mM-P_i. Further additions were as described in the legend to Fig. 1.

Swelling of the mitochondria was determined by incubating the particles, at approx. 1 mg of protein/ml, in the medium of the Co-deuteroporphyrin synthesis experiments, or in the medium of the polarographic experiments (see above). CoCl₂ (5–100 μ M) was added and the change in absorbance at 520 nm was recorded.

Protein was determined by the Folin-Ciocalteu reagent (Flatmark *et al.*, 1971). Malate dehydrogenase (EC 1.1.1.37) was determined according to Bergmeyer (1974). All spectrophotometric measurements were performed on an Aminco DW-2 u.v./vis. spectrophotometer, band-width 3 nm.

Chemicals

ADP, albumin (bovine, essentially fatty-acid free), haemin (bovine, crystalline, type I), Hepes (A grade) and rotenone were the products of the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Human albumin, highly purified, was obtained from Kabi, Stockholm, Sweden. Deuteroporphyrin IX was purchased from Porphyrin Products (Logan, UT, U.S.A.). $^{57}\text{CoCl}_2$ was obtained from The Radiochemical Centre (Amersham, Bucks., U.K.). Globin (undenatured) was purchased from ICN Pharmaceuticals (Cleveland, OH, U.S.A.).

Other chemicals were of highest purity commercially available. Double quartz-distilled water was used throughout.

The purity of deuteroporphyrin was determined by high-pressure liquid chromatography (Gray *et al.*, 1977). A μ Porasil (300 mm $\times$ 4 mm) column was used with a Waters Associates model 6000 solvent-delivery system, a U6K injector and a model 440 absorbance detector (filter 405 nm). The elution solvent was n-heptane/methyl acetate (3:2, v/v) at a flow rate of 1.5 ml/min and a pressure drop of 500 p.s.i. (3.45 MPa). More than 97% of the absorbing material was found as a single peak with an elution volume different from protoporphyrin ester and mesoporphyrin ester.

Prior to use, globin was run through a column of Sephadex G-50 equilibrated and eluted with 10 mM-

Hepes, pH 7.40. Albumin was used without further purification.

The purity of globin and albumin was determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (Brewer *et al.*, 1974). The gels were scanned on a Beckman CDS-100 computing densitometer, slit 0.4 mm $\times$ 5 mm, wavelength 520 nm. More than 97% of the stainable proteins were localized in a single band.

The purity of haemin was determined from the ratio: absorbance at the α -maximum/absorbance at the minimum between the α -band and the β -band (Fuhrhop & Smith, 1975). The preparations used were more than 95% pure.

Results

From the localization of the ferrochelatase (Jones & Jones, 1969) it follows that, to be physiologically relevant, studies on the efflux of metalloporphyrins should be limited to mitochondria with an intact membrane system and with preserved functional integrity. In the present study only mitochondria with respiratory control ratios for ADP > 4 at the start of the experiments were used, and the ratios were determined at intervals during the incubation period. Moreover, since deuteroporphyrin (Koller & Romslo, 1978) as well as protoporphyrin (Stumpf *et al.*, 1979) are potent uncoupling agents, the porphyrin substrate was added at a concentration about one-fifth of that necessary for uncoupling.

Synthesis and efflux of Co-deuteroporphyrin

When isolated rat-liver mitochondria were incubated with $^{57}\text{CoCl}_2$ plus deuteroporphyrin at concentrations of $8\mu\text{M}$ and $2.0\mu\text{M}$ respectively, the amount of ^{57}Co recovered in the cyclohexanone phase increased in a sigmoidal fashion as a function of time, essentially as reported for the formation of iron-deuteroporphyrin (Koller *et al.*, 1976). Thus, after a lag phase of 3–6 min a steady-state rate of approx. 80 pmol of Co-deuteroporphyrin/min per mg of protein was obtained. When about 85% of the deuteroporphyrin had been consumed, the rate of Co-deuteroporphyrin synthesis levelled off and during the following 20–30 min there was a slight decline in the amount of radioactivity recovered in the organic phase.

The efflux of Co-deuteroporphyrin remained relatively constant at about 10% of the total amount of Co-deuteroporphyrin synthesized during the first 10–15 min of incubation. Thereafter the efflux rapidly increased to reach a second steady-state level of 25–35% of the total amount of Co-deuteroporphyrin synthesized at 30–45 min (Fig. 1). The increase in the efflux coincided with loose coupling of the mitochondria (Fig. 1); i.e. Co-deuteroporphyrin leaked to the environment at a rate

apparently inversely proportional to the functional intactness of the particles (Fig. 2).

Effect of albumin, globin, haemin and haemoglobin on Co-deuteroporphyrin synthesis and efflux

Albumin binds free fatty acids and calcium and thereby protects mitochondria from uncoupling (Scarpa & Lindsay, 1972). Accordingly, the efflux of Co-deuteroporphyrin in the presence of albumin

Fig. 1. Time progress curves for the release of Co-deuteroporphyrin to the supernatant (●) and the decrease in respiratory control value (◇) of isolated rat-liver mitochondria

Mitochondria were incubated with deuteroporphyrin and CoCl_2 (see the Materials and Methods section). Rotenone ($1.5\mu\text{M}$) was added, and at the time intervals indicated State-4 respiration was initiated by adding succinate, followed after 3 min by ADP at final concentrations of 3 mM and 8 mM respectively. In a parallel sample the synthesis of Co-deuteroporphyrin and the Co-deuteroporphyrin released to the supernatant (%) was determined (see the Materials and Methods section).

Fig. 2. Relationship between the respiratory control value of the mitochondria and the release of Co-deuteroporphyrin to the supernatant after 10 min

The results from 15 different mitochondrial preparations are presented.

would be expected to be below that of a control without albumin, as actually found (Fig. 3a). However, when the incubation is extended beyond 40 min the efflux in the presence of albumin surpasses that of a control without albumin. The rate of Co-deuteroporphyrin synthesis in the presence of albumin is below that of a control without albumin (Fig. 3b). We interpret the results to mean that albumin competes with the mitochondria for the binding of the porphyrin substrate (Fig. 3b and Koller, 1979), and also stabilizes the functional integrity of the mitochondria and thereby decreases the efflux of Co-deuteroporphyrin during the initial 20 min of incubation (Fig. 2). Following uncoupling and leakage of Co-deuteroporphyrin, albumin binds the metalloporphyrin with high affinity and enhances the efflux.

Human serum albumin binds porphyrins and metalloporphyrins with higher affinity than bovine serum albumin (Israels *et al.*, 1975). Accordingly, the rate and the total amount of Co-deuteroporphyrin synthesized in the presence of human serum albumin is below that synthesized in the presence of bovine serum albumin and the efflux following uncoupling is higher (Table 1). The efflux following prolonged incubation with human serum albumin (Table 1) is very close to the figures given by Israels *et al.* (1975) with protohaem and human albumin.

Globin behaves quantitatively and qualitatively differently from albumin. Thus, globin has no effect either on the integrity of the mitochondria (data not shown) or on the rate or the total amount of Co-deuteroporphyrin synthesized (Fig. 3b). On the other hand, the efflux of Co-deuteroporphyrin is markedly enhanced at a time when the mitochondria are still tightly coupled (Fig. 3a). This effect is seen at concentrations of the $\alpha\beta$ -globin dimer $>0.4\mu\text{M}$ (Figs. 3a, 4a). When haemin is added together with globin, the effect of globin on the efflux of Co-deuteroporphyrin is inhibited in a competitive manner (Fig. 5).

Haemoglobin at concentrations up to $0.5\mu\text{M}$ has no effect either on the synthesis or on the efflux of Co-deuteroporphyrin (data not shown).

By contrast, exogenously added haemin markedly depresses the efflux of endogenously synthesized

Fig. 3. Time progress curve for the release of Co-deuteroporphyrin to the supernatant (a) and synthesis of Co-deuteroporphyrin (b)

Experimental conditions were as described (see the Materials and Methods section). ●, ○, Incubation medium alone; ■, □, incubation medium supplemented with 4 μM-albumin; ▲, △, incubation medium supplemented with 4 μM-globin (calculated as $\alpha\beta$ -globin dimer).

Table 1. Effect of bovine and human serum albumin on the synthesis and efflux of Co-deuteroporphyrin from isolated rat-liver mitochondria

Mitochondria, about 2 mg of protein/ml, were incubated as described (see the Materials and Methods section). The amount of ⁵⁷Co-deuteroporphyrin synthesized and the release of ⁵⁷Co-deuteroporphyrin to the supernatant (%) were determined after 10 and 60 min. The concentration of albumin was 4 μM. The results are the means and the ranges (in parentheses) from seven separate experiments.

Addition	Co-deuteroporphyrin synthesis (pmol/mg of protein) after		Co-deuteroporphyrin release to supernatant (%) after	
	10 min	60 min	10 min	60 min
None (control)	381 (329–505)	960 (675–1209)	10.5 (5.0–16.0)	24.9 (20.0–33.0)
Bovine albumin	154 (104–266)	695 (487–831)	8.4 (7.0–11.0)	32.9 (27.0–43.0)
Human albumin	53 (36–86)	361 (197–590)	11.2 (4.0–17.0)	70.5 (62.0–81.0)

Fig. 4. Effect of increasing concentrations of albumin and globin on the release of Co-deuteroporphyrin to the supernatant (a) and the synthesis of Co-deuteroporphyrin (b)

Experimental conditions were as described (see the Materials and Methods section). The incubation medium was supplemented with globin (▲, △) or albumin (■, □). Synthesis and efflux of Co-deuteroporphyrin were determined at 10 min (□, △) and 60 min (■, ▲).

Co-deuteroporphyrin (Fig. 6); the inhibition is apparent at concentrations significantly below those necessary to depress the ferrochelatase (Koller & Romslo, 1977; Jones & Jones, 1970), and the uptake of porphyrins (Koller, 1979).

Discussion

Cobalt and deuteroporphyrin are not the physiological substrates for the metal-chelatase. There is, however, considerable evidence to support their use as suitable model compounds. In ferrochelatase assays they behave qualitatively essentially as the natural substrates iron and protoporphyrin IX (Jones & Jones, 1969; Jones & Jones, 1970; Barret & Jones, 1978; Koller *et al.*, 1976). Cobalt is readily incorporated into protoporphyrin IX *in vivo* (Sinclair *et al.*, 1979). The metalloporphyrin thus formed enhances globin synthesis (Jackson &

Fig. 5. Effect of haemin on the globin-stimulated efflux of Co-deuteroporphyrin

Mitochondria were incubated as described (see the Materials and Methods section) in the presence of increasing concentrations of globin. O, Incubation medium; ●, incubation medium supplemented with 0.5 μM-haemin; ●, incubation medium supplemented with 2.0 μM-haemin. The reaction was terminated at 10 min and the efflux of Co-deuteroporphyrin (%) was determined.

Fig. 6. Effect of increasing concentrations of haemin on the synthesis of Co-deuteroporphyrin and on the release of Co-deuteroporphyrin to the supernatant

Experimental conditions were as described (see the Materials and Methods section). The incubation medium was supplemented with haemin, and the reaction was terminated at 10 min. The results are given as values (%) relative to the results in the absence of haemin. ●, Co-deuteroporphyrin synthesized; O, Co-deuteroporphyrin released to the medium.

Hunter, 1970) and it inhibits aminolaevulinate synthetase activity in a fashion analogous to protohaem (Bonkowsky *et al.*, 1979), i.e. Co-proto-

porphyrin formed within the mitochondria escapes to the cytosol. Deuteroporphyrin is readily taken up by the mitochondria (Koller & Romslo, 1978), and it is a well-suited substrate to the ferrochelatase in whole cells (Jackson & Hunter, 1970), bacteria (Barret & Jones, 1978) and isolated mitochondria (Koller *et al.*, 1976).

Experimentally, cobalt and deuteroporphyrin offer several advantages compared to iron and protoporphyrin IX. Cobalt is soluble at neutral pH, it has no effect either on the respiratory activity or on the swelling properties of the mitochondria (data not shown) and it reacts with deuteroporphyrin with great avidity. For comparison, with iron we are faced with the problems of its extreme insolubility around neutrality, the noxious effect of iron on mitochondrial metabolism (Romslo, 1975) and the ignorance as to the nature of the iron-donor to mitochondria *in situ* (Jacobs, 1977; Speyer & Fielding, 1979). Deuteroporphyrin is more easily soluble at neutral pH than protoporphyrin IX and it reacts with the metal at a rate significantly above that of protoporphyrin IX (Labbe *et al.*, 1963; Jones & Jones, 1970; Honeybourne *et al.*, 1979). Thus, with cobalt and deuteroporphyrin relative to iron and protoporphyrin IX, only quantitative differences in the rate and the amount of metalloporphyrins synthesized would be expected.

Co-deuteroporphyrin is more water-soluble than protohaem at neutral pH, and it would therefore be distributed more in the incubation medium than in the mitochondria if the efflux was by passive diffusion. As shown here, however, Co-deuteroporphyrin does not distribute by passive diffusion. Indeed, it is actively retained by the mitochondria (Figs. 1 and 2). The efflux of Co-deuteroporphyrin, both in absolute and relative terms, is inversely correlated to the energy-coupling of the mitochondria, i.e. the ability of the mitochondria to maintain a concentration gradient of Co-deuteroporphyrin depends on metabolic energy.

The effect of globin and albumin undoubtedly reflects binding of the porphyrin substrate (Yoda & Israels, 1972; Ponka *et al.*, 1973; Israels *et al.*, 1975; Koller, 1979) and the metalloporphyrin product (Winterhalter *et al.*, 1969; Schulman, 1974; Müller-Eberhard & Morgan, 1975). However, the results cannot be explained solely by differences in association constants. The association constants for the deuterohaem- or deuteroporphyrin-albumin complexes are of the order of 10^6 – 10^8 l/mol (Müller-Eberhard & Morgan, 1975; Müller-Eberhard & Liem, 1978) and for the corresponding globin complexes 10^{12} – 10^{15} l/mol (Banerjee, 1962; Gibson & Antonini, 1963; Schulman, 1974). Thus, compared to albumin, globin should be a more effective inhibitor of the uptake of porphyrins and also it should more effectively trap metalloporphyrin

released to the medium. Albumin, however, is by far the most effective inhibitor of the uptake of porphyrins (Koller, 1979) and of metalloporphyrin synthesis (Fig. 4b) whereas globin is the most effective metalloporphyrin-mobilizing compound (Fig. 4a).

If globin and albumin functioned only by trapping metalloporphyrin, it would be expected that, as the absolute amount of Co-deuteroporphyrin inside the mitochondria increased with time (see Fig. 3b), the transmembrane concentration gradient would also increase, as would the rate of efflux. Apparently this reasoning applies to albumin, at least following uncoupling (Fig. 3a). Globin, however, operates at a much higher efficiency and at a much lower transmembrane Co-deuteroporphyrin concentration gradient, and its effect is not increased at higher concentrations of Co-deuteroporphyrin within the mitochondria. A challenging hypothesis would be that in contrast to albumin, $\alpha\beta$ -globin dimers penetrate the inner membrane, bind to metalloporphyrin, and escape to the medium.

That apo-haemoproteins may interact with mitochondrial membranes has recently been shown by Korb & Neupert (1978). Apo-(cytochrome *c*) combines with its prosthetic group at the mitochondrial-cytosolic interphase; the complete holoenzyme becomes trapped within the membrane. It has also been shown that a series of high-molecular-weight proteins of microsomal origin, e.g. aspartate aminotransferase (Marra *et al.*, 1977; Waksman *et al.*, 1977), malate dehydrogenase (Waksman *et al.*, 1977) and aminolaevulinate synthetase (Ohasi & Sinohara, 1978) penetrate to within the inner membrane and the matrix space. Thus, it would not be unexpected that the $\alpha\beta$ -globin dimer of molecular weight approx. half that of aminolaevulinate synthetase (Ohasi & Sinohara, 1978) would penetrate the inner membrane. The concept of globin as a physiological promoter of haem efflux from the mitochondria is also supported by studies from other laboratories. In rabbit reticulocytes, ferrihaemoglobin stimulated the synthesis of $\alpha\beta$ -globin dimers, which then readily removed haem from ferrihaemoglobin (Schulman, 1974); when reticulocytes were treated with cycloheximide to inhibit globin synthesis, haem accumulated in the mitochondria (Neuwirt *et al.*, 1972; Ponka *et al.*, 1973).

The high affinity of haem for various proteins, surfaces and membranes makes it unlikely that there is a free haemin pool, in the sense of unassociated molecules, in the cytosol (Granick & Beale, 1978); there is rather a pool of uncommitted, not unattached, haem (Granick & Beale, 1978). In reticulocytes the unattached haemin pool is of the order of 10^{-6} – 10^{-5} mol/l (Neuwirt *et al.*, 1972). In hepatocytes the concentration of unattached haemin is not known. It should be noted, however, that the effect

of haemin on the efflux of metalloporphyrin (Fig. 6) is found at concentrations far below those necessary to evoke stimulation of globin synthesis (Bruns & London, 1965), to decrease the release of iron from transferrin (Ponka & Neuwirt, 1969) and to inhibit the ferrochelatase (Jones & Jones, 1970) of intact mitochondria and of sonicated mitochondria (Koller & Romslo, 1977).

The physiological significance of the present results remains to be assessed. It is, however, unlikely that the efflux process, regulated as it is by the mitochondrial energy state and the presence of globin and haemin in the incubation medium, should be a finding *in vitro* only.

The technical assistance of Mr. T. Bjørndal is greatly acknowledged. The study was supported in part by the Norwegian Research Council for Science and the Humanities.

References

- Banerjee, R. (1962) *Biochim. Biophys. Acta* **64**, 368–384
- Barret, J. & Jones, O. T. G. (1978) *Biochem. J.* **174**, 277–281
- Bergmeyer, H. U. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 613–617, Academic Press, New York
- Bonkowski, H. L., Sinclair, P. R. & Sinclair, J. F. (1979) *Yale J. Biol. Med.* **52**, 13–37
- Brewer, J. M., Pesce, A. J. & Ashworth, R. B. (1974) in *Experimental Techniques in Biochemistry*, pp. 128–160, Prentice-Hall, Englewood Cliffs, NJ
- Bruns, G. P. & London, I. M. (1965) *Biochem. Biophys. Res. Commun.* **18**, 236–242
- Davidian, N. M. & Penniall, R. (1971) *Biochem. Biophys. Res. Commun.* **44**, 15–21
- Felicitelli, L., Colombo, B. & Baglioni, C. (1966) *Biochim. Biophys. Acta* **129**, 380–394
- Flatmark, T., Terland, O. & Helle, K. B. (1971) *Biochim. Biophys. Acta* **226**, 9–19
- Fuhrhop, J. H. & Smith, K. M. (1975) in *Laboratory Methods in Porphyrin and Metalloporphyrin Research*, p. 50, Elsevier, Amsterdam
- Garner, R. C. & McLean, A. E. M. (1969) *Biochem. Biophys. Res. Commun.* **37**, 883–887
- Gibson, Q. H. & Antonini, E. (1963) *J. Biol. Chem.* **238**, 1384–1388
- Granick, S. & Beale, S. I. (1978) *Adv. Enzymol.* **41**, 33–201
- Gray, C. H., Lim, C. K. & Nicholson, D. C. (1977) *Clin. Chim. Acta* **77**, 167–178
- Higashi, T., Kawamata, F. & Sakamoto, T. (1974) *J. Biochem. (Tokyo)* **76**, 703–708
- Honeybourne, C. L., Jackson, J. T. & Jones, O. T. G. (1979) *FEBS Lett.* **98**, 207–210
- Israels, L. G., Yoda, B. & Schachter, B. A. (1975) *Ann. N.Y. Acad. Sci.* **244**, 651–661
- Jackson, R. & Hunter, T. (1970) *FEBS Lett.* **9**, 61–63
- Jacobs, A. (1977) *Blood* **50**, 433–439
- Jones, M. S. & Jones, O. T. G. (1969) *Biochem. J.* **113**, 507–514
- Jones, M. S. & Jones, O. T. G. (1970) *Biochem. Biophys. Res. Commun.* **41**, 1072–1079
- Ketterer, B., Singh Srai, K. & Christodoulides, L. (1976) *Biochim. Biophys. Acta* **428**, 683–689
- Koller, M.-E. (1979) *FEBS Lett.* **100**, 47–51
- Koller, M.-E. & Romslo, I. (1977) *Biochim. Biophys. Acta* **461**, 283–296
- Koller, M.-E. & Romslo, I. (1978) *Biochim. Biophys. Acta* **503**, 238–250
- Koller, M.-E., Romslo, I. & Flatmark, T. (1976) *Biochim. Biophys. Acta* **449**, 480–490
- Korb, H. & Neupert, W. (1978) *Eur. J. Biochem.* **91**, 609–620
- Labbe, R. F., Hubbard, N. & Caughey, W. S. (1963) *Biochemistry* **2**, 372–374
- Marra, E., Doonan, S., Saccone, C. & Quagliarillo, E. (1977) *Biochem. J.* **164**, 685–691
- Marver, H., Tschudy, D. P., Perloroth, M. G. & Collins, A. (1966) *Science* **153**, 501–503
- Marver, H. S. (1969) in *Microsomes and Drug Oxidations* (Gilette, J. R., Conney, A. H., Cosmides, G. J., Eastabrook, R. W., Fouts, J. R. & Mannering, G. J., eds.), pp. 495–511, Academic Press, New York
- Morgan, W. T. (1976) *Ann. Clin. Res.* **8**, 223–232
- Müller-Eberhard, U. & Liem, H. H. (1978) in *Diagnosis and Therapy of Porphyrrias and Lead Intoxication* (Doss, M., ed.), pp. 272–275, Springer-Verlag, Berlin, Heidelberg, New York
- Müller-Eberhard, U. & Morgan, W. T. (1975) *Ann. N.Y. Acad. Sci.* **244**, 624–650
- Neuwirt, J., Ponka, P. & Borowa, J. (1972) *Biochim. Biophys. Acta* **264**, 235–244
- Ohasi, A. & Sinohara, H. (1978) *Biochem. Biophys. Res. Commun.* **84**, 76–82
- Ponka, P. & Neuwirt, J. (1969) *Blood* **33**, 690–706
- Ponka, P., Borova, J. & Neuwirt, J. (1973) *Biochim. Biophys. Acta* **304**, 715–718
- Romslo, I. (1975) *Biochim. Biophys. Acta* **387**, 69–79
- Romslo, I. & Flatmark, T. (1973a) *Biochim. Biophys. Acta* **305**, 29–40
- Romslo, I. & Flatmark, T. (1973b) *Biochim. Biophys. Acta* **325**, 38–46
- Scarpa, A. & Lindsay, J. G. (1972) *Eur. J. Biochem.* **27**, 401–407
- Schiefer, H. G. (1969) *Hoppe-Seyler's Z. Physiol. Chem.* **350**, 921–928
- Schulman, H. M. (1974) *Can. J. Biochem.* **52**, 665–669
- Sinclair, P., Gibbs, A. H., Sinclair, J. F. & DeMatteis, F. (1979) *Biochem. J.* **178**, 529–538
- Speyer, B. E. & Fielding, J. (1979) *Br. J. Haematol.* **42**, 255–267
- Stumpf, D. A., McCabe, E. R. B., Parks, J. K., Bullen, W. W. & Schiff, S. (1979) *Biochem. Med.* **21**, 182–189
- Tipping, E., Ketterer, B., Christodoulides, L. & Enderby, G. (1976) *Biochem. J.* **157**, 461–467
- Waksman, A., Rendon, A., Cremel, G., Pellicone, C. & Goubault de Brugiere, J. F. (1977) *Biochemistry*, **16**, 4703–4707
- Winterhalter, K. H., Heywood, J. D., Huehns, E. R. & Finch, C. A. (1969) *Br. J. Haematol.* **16**, 523–535
- Yoda, B. & Israels, L. G. (1972) *Can. J. Biochem.* **50**, 633–637