Studies on the efflux of metalloporphyrin from rat liver mitochondria*

Effect of K⁺ and other cations

Paul HUSBY and Inge ROMSLO

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

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The mechanism by which metalloporphyrins synthesized within the mitochondria escape to the incubation medium was studied in isolated rat liver mitochondria. In a low-ionic-strength sucrose medium, the efflux of metalloporphyrins is markedly decreased when K⁺ (> 10 mM) is added. The effect of K⁺ is not dependent on the energy state of the mitochondria and it can in part be abolished by adding globin, but not albumin. K⁺ also decreases the uptake of porphyrins by the mitochondria and thereby the rate of synthesis of metalloporphyrins. Qualitatively similar results are found with Na⁺, Li⁺, Mg²⁺ and Ca²⁺. Quantitatively, however, the efficiency of cations to inhibit the release of metalloporphyrins decreases in the order: Mg²⁺ > Ca²⁺ > K⁺ > Li⁺ > Na⁺. Co-protoporphyrin behaves essentially as Co-deutero porphyrin. The results provide further evidence that the efflux of metalloporphyrins from the mitochondria depends on haem-binding ligands of the suspending medium and also on the ionic strength of the incubation medium.

The mitochondrial inner membrane as a permeability barrier to the transport of haem-biosynthesis intermediates is well known (Sano & Granick, 1961; Jones & Jones, 1969). Only recently, however, have the mechanisms for the transport of δ -aminolaevulinic acid (Jones & Jones, 1970), iron (Romslo & Flatmark, 1973a; Flatmark & Romslo, 1975) and porphyrin (Koller & Romslo, 1978, 1980) been worked out in more detail.

The release of metalloporphyrins from the mitochondria is less well understood. Studies on isolated mitochondria have shown that the efflux of metalloporphyrin increases inversely with the energy state of the particles (Husby & Romslo, 1980) and it increases also in the presence of suitable metalloporphyrin-binding ligands of the medium (Yoda & Israels, 1972; Ponka *et al.*, 1973; Israels *et al.*, 1975; Taketani & Tokunaga, 1980; Husby & Romslo, 1980; Husby *et al.*, 1980).

The uptake by the mitochondria of respiratory substrates (Meisner *et al.*, 1972), ADP (Meisner, 1971; Krämer, 1980), iron (Romslo, 1975) and porphyrins (Koller & Romslo, 1978) is markedly influenced by the ionic composition of the medium,

in particular the concentration of Mg²⁺ and K⁺. The effect of cations on the efflux of metalloporphyrins is not known. It would be expected, however, that factors that affect the uptake of porphyrins (and iron) may have some impact on the efflux of metalloporphyrins as well, particularly if the transport processes are by a coupled shuttle mechanism.

The present paper deals with the effect of mono- and bi-valent cations on the synthesis and the efflux of metalloporphyrins from isolated rat liver mitochondria.

Materials and methods

Rat liver mitochondria were prepared as described by Romslo & Flatmark (1973b). The functional integrity of the preparations was tested by measuring the respiratory-control ratio with ADP, succinate being used as the substrate. Only mitochondria with this ratio at a value of more than 4 at the start of the experiments were used.

Deterioration of the mitochondria during incubation was determined from the leakage of malate dehydrogenase and the decrease in respiratory-control ratio (Husby & Romslo, 1980). Less than 1% of the malate dehydrogenase activity of the mitochondria leaked to the incubation medium during 60 min incubation, and the mitochondria

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

* The present paper is the second in a series on this topic; the first is Husby & Romslo (1980).

remained coupled for about 20 min in the absence as well as in the presence of K^+ (see below and Fig. 4).

Submitochondrial particles were prepared by sonicating the mitochondrial suspension at 4°C [three 30 s bursts in an MSE 150 W ultrasonic disintegrator (model MK 2) operated with 9.5 mm-diameter end probe and an amplitude reading of 18 μ m].

Uptake of deuteroporphyrin

Mitochondria (about 1.5 mg of protein) were incubated at 30°C in a final volume of 1 ml of 0.25 M-sucrose/5 mM-Hepes buffer, pH 7.40, or 1 ml of 0.06 M-sucrose/5 mM-Hepes buffer (pH 7.40)/100 mM- Na^+ (Li^+ or K^+).

The reaction was initiated by adding 5 μ l of 2.4 mM-deuteroporphyrin (final concentration 12 μ M-deuteroporphyrin). At 30 s the accumulation of deuteroporphyrin was terminated by adding 0.5 ml of ice-cooled buffer, followed by immediate centrifugation in an Eppendorf microcentrifuge (type 3200) for 2 min at 13 000 g.

The amount of deuteroporphyrin accumulated was determined from the decrease in the fluorescence of the supernatant after centrifugation.

The fluorescence was measured in a Jasco FP-4 fluorescence spectrophotometer. Excitation and emission wavelengths were 393 and 593 nm respectively. The spectral bandwidths of the excitation and the emission monochromators were 10 nm.

Synthesis and release of metalloporphyrins

Synthesis and release of metalloporphyrins were determined as described by Husby & Romslo (1980). The concentrations of deuteroporphyrin and protoporphyrin were 2 μ M and 8 μ M respectively.

Other analytical procedures

Mitochondrial respiration rates were determined as previously described (Husby & Romslo, 1980).

Protein was determined with the Folin-Ciocalteu reagent (Flatmark *et al.*, 1971).

Chemicals

ADP, rotenone and Hepes (A-grade) were the products of Sigma Chemical Co. (St. Louis, MO, U.S.A.). Deuteroporphyrin IX and protoporphyrin IX were purchased from Porphyrin Products (Logan, UT, U.S.A.). $^{57}CoCl_2$ was obtained from The Radiochemical Centre (Amersham, Bucks., U.K.).

The purity of the porphyrins was determined as described by Husby & Romslo (1980).

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

Results

An objection to our results on Co-porphyrin efflux could be that Co-deuteroporphyrin may leak into the medium during incubation and, owing to its poor solubility in water, co-precipitates with the mitochondria during the subsequent centrifugation. However, if so, it is hard to explain why albumin,

Fig. 1. Effect of centrifugation on the distribution of ^{57}Co -deuteroporphyrin generated within mitochondria (a) Mitochondria (2 mg of protein/ml) were incubated as described in the Materials and methods section by Husby & Romslo (1980). The concentration of deuteroporphyrin was 2 μ M and of $^{57}CoCl_2$, 8 μ M. The distribution of ^{57}Co -deuteroporphyrin in the mitochondria (P) and the supernatant (S) was determined at 10 min. (b) Mitochondria (about 2 mg of protein/ml) were incubated at 30°C in a medium containing 0.25 M-sucrose, 5 mM-Hepes buffer, pH 7.40, 4.75 μ M-deuteroporphyrin and 16 μ M- $^{57}CoCl_2$ (30 000 c.p.m./nmol). After 60 min incubation, portions (1.5 ml) were withdrawn and centrifuged in an Eppendorf microcentrifuge (type 3200) for 2 min at 13 000 g. The supernatant was mixed with an equal volume of 0.15 M-sucrose, 5 mM-Hepes buffer, pH 7.40, 5 μ M-albumin and 50 mM-KCl. The concentration of ^{57}Co -deuteroporphyrin was 1.6 μ M. Mitochondria (2 mg of protein/ml) were added and, after 10 min incubation, portions were withdrawn and centrifuged (see above). The distribution of ^{57}Co -deuteroporphyrin in the supernatant (S) and the pellet (P), was determined (see the Materials and methods section).

which binds Co-deuteroporphyrin essentially in the same way as hemopexin (Müller-Eberhard, 1978; Müller-Eberhard & Morgan, 1975), does not enhance the efflux of Co-deuteroporphyrin (Husby & Romslo, 1980). Indeed, at concentrations of albumin in excess of $5\text{ }\mu\text{M}$, the efflux of Co-deuteroporphyrin actually decreases (Husby & Romslo, 1980). More direct evidence against the view that our method measures only co-precipitation of metalloporphyrins are the results given in Figs. 1 and 2. Added ^{57}Co -deuteroporphyrin incubated together with albumin and mitochondria in a K^+ -rich medium distributes to approx. 90% in the supernatant, whereas ^{57}Co -deuteroporphyrin generated by the mitochondria in a similar medium is found to approx. 10% in the supernatant (Fig. 1). Moreover, if distributed by partition only, the concentration of Co-deuteroporphyrin in the pellet would be expected to decline with increasing dilution. However, increasing the dilution 40-fold has no effect on the pellet Co-deuteroporphyrin (Fig. 2). Our method therefore does measure metalloporphyrin efflux, not merely partition of metalloporphyrin between the hydrophobic membrane and the incubation medium.

Effect of K^+

The release of Co-deuteroporphyrin from the mitochondria is markedly influenced by the con-

centration of K^+ of the incubation medium. This relates to the release with increasing concentrations of K^+ at constant time (Fig. 3) and to the release with time at constant K^+ (Fig. 4). Thus at 10 min

Fig. 3. Effect of increasing concentrations of K^+ on the release of Co-deuteroporphyrin to the supernatant.

Experimental conditions were as described in the Materials and methods section. The osmolality was kept constant at 270 mosmol/kg by varying the concentration of sucrose. ●, 10 min incubation; ▲, 60 min incubation.

Fig. 2. Effect of dilution on the release of ^{57}Co -deuteroporphyrin from mitochondria

Mitochondria (2 mg of protein/ml) were incubated as described in the Materials and methods section. The concentration of deuteroporphyrin was $2\text{ }\mu\text{M}$ and of $^{57}\text{CoCl}_2$, $8\text{ }\mu\text{M}$. At 10 min, 1 ml portions were withdrawn and taken up in increasing volumes of ice-cold incubation buffer. The amount of pelleted ^{57}Co -deuteroporphyrin was determined after centrifugation at $10\,000\text{ g}$ for 10 min.

Fig. 4. Time-progress curves for the release of Co-deuteroporphyrin to the supernatant (○, ●), the decrease in respiratory control value (□, ■) of isolated rat liver mitochondria in a K^+ -depleted medium (○, □) and in a medium supplemented with 100 mM-K^+ (●, ■)

The osmolality was 270 mosmol/kg. Mitochondria were incubated with deuteroporphyrin and CoCl_2 (see the Materials and methods section). Rotenone ($1.5\text{ }\mu\text{M}$) was added, and at the time intervals indicated, State-4 respiration was initiated by adding 5 mM-P_i , 5 mM-Mg^{2+} and 3 mM-succinate , followed after 3 min by 8 mM-ADP . In a parallel sample the synthesis of Co-deuteroporphyrin released to the supernatant (%) was determined (see the Materials and methods section).

incubation there is a slight decrease in the release of Co-deuteroporphyrin when the concentration of K^+ is increased from zero to 100mM, and at 60min incubation the release of Co-deuteroporphyrin decreases from 30% in a K^+ -depleted medium to about 10% at $K^+ > 25$ mM (Fig. 3). With time the release of Co-deuteroporphyrin remains at 5–10% in a medium of 100mM- K^+ even though the mitochondria are largely uncoupled (Fig. 4). By contrast, in a K^+ -depleted medium during the first 10–20min of incubation the release remains at about 10%. Thereafter the efflux rapidly increases to reach a value of about 25–35% of the total amount of Co-deuteroporphyrin synthesized at 35–45 min. The increase in the efflux coincides with loose coupling of the mitochondria (Husby & Romslo, 1980; the present Fig. 4). The effect of K^+ cannot therefore be through preservation of the functional integrity of the mitochondria.

K^+ at concentrations greater than 10–20mM decreases not only the release of metalloporphyrin from the mitochondria, but also the uptake of porphyrin (Koller & Romslo, 1978; the present Table 1) as well as of iron (Romslo, 1975).

The metal-chelataze reaction of sonicated mitochondria is not inhibited by K^+ at concentrations in excess of 100mM (the present Fig. 5; Koller & Romslo, 1980). The inhibitory effects of high K^+ concentrations on Co-deuteroporphyrin synthesis (Fig. 6) therefore most likely reflect substrate depletion subsequent to the decreased uptake of porphyrin. After 60min incubation, however, the amount of metalloporphyrin synthesized is essentially the same whether K^+ is added or not.

Globin has been shown to enhance the mobilization of metalloporphyrin from mitochondria in a K^+ -depleted medium, and the effect is not through loose coupling (Husby & Romslo, 1980). In the presence of K^+ the effect of globin is largely decreased (Table 2). At concentrations of K^+ in excess of 10mM, the release of Co-deuteroporphyrin is decreased by 40–50% even though the concentration of globin is increased from 1.7 μ M to

Fig. 5. Time-progress curves for Co-deuteroporphyrin synthesis by submitochondrial particles

Submitochondrial particles were prepared as described in the Materials and methods section. Submitochondrial particles (about 4 mg of protein/ml) were incubated in a K^+ -depleted medium (○) or in a medium containing 100mM- KCl (●). $CoCl_2$ and deuteroporphyrin were added at final concentrations of 8 μ M and 2 μ M respectively.

Fig. 6. Time-progress curves for Co-deuteroporphyrin synthesis by isolated rat liver mitochondria incubated in a K^+ -depleted medium (□) or in a medium containing 10mM- (○) or 50mM- (△) KCl

Mitochondria (about 2mg of protein/ml) were incubated as described in the Materials and methods section.

Table 1. Effect of univalent cations on the uptake of deuteroporphyrin by isolated rat liver mitochondria. Mitochondria (about 1.5mg of protein/ml) were incubated as described in the Materials and methods section. The results are the means and the ranges (in parentheses) from three separate experiments.

Addition	(mM)	Deuteroporphyrin uptake (nmol/mg of protein)
Control	—	6.8 (5.3–8.0)
K^+	100	1.8 (1.7–2.1)
Na^+	100	2.6 (2.4–2.8)
Li^+	100	2.3 (1.8–2.6)

Table 2. *Effect of globin on the release of Co-deuteroporphyrin from isolated rat liver mitochondria in the absence and presence of K⁺*

Mitochondria (about 2 mg of protein/ml) were incubated as described in the Materials and methods section. The osmolality was kept constant at 270 mosmol/kg of solution by varying the concentration of sucrose. The results are the means and the ranges (in parentheses) from three separate experiments.

K ⁺ (mM)	Globin (μM)	Co-deuteroporphyrin released to the supernatant (%) after:	
		10 min	60 min
—	—	10 (6–12)	28 (25–30)
—	1.7	65 (55–83)	83 (79–90)
—	4.5	79 (72–88)	100 (97–100)
10	—	9 (7–12)	11 (7–15)
10	1.7	55 (50–60)	58 (48–70)
10	4.5	56 (52–60)	58 (58–59)
10	8.5	48 (42–54)	60 (55–62)
5	—	10 (8–14)	22 (20–25)
5	1.7	69 (57–88)	95 (92–98)
10	1.7	48 (38–57)	58 (48–70)
25	1.7	49 (44–53)	48 (42–57)
50	1.7	47 (45–47)	50 (48–60)

Table 3. *Effect of Na⁺, Li⁺ and K⁺ on the rate of Co-deuteroporphyrin synthesis by isolated rat liver mitochondria*

Mitochondria (about 2 mg of protein/ml) were incubated as described in the Materials and methods section. The osmolality was kept constant at 270 mosmol/kg by varying the concentration of sucrose. The results are the means and the ranges (in parentheses) from eight experiments. The concentration of the cations was 100 mM. The rates were calculated from the time–progress curves between 5 and 10 min of incubation.

Addition	Co-deuteroporphyrin synthesis (pmol/min per mg of protein)
Control	76 (59–101)
Na ⁺	60 (51–81)
Li ⁺	28.5 (25–32)
K ⁺	17.5 (15–22)

8.5 μM. On the other hand, at 1.7 μM-globin the release of Co-deuteroporphyrin cannot be decreased below 40–50% by increasing the K⁺ concentration from 10 to 50 mM. By comparison, albumin, which mobilizes metalloporphyrin only from uncoupled mitochondria (Husby & Romslo, 1980), has no effect on the efflux of Co-deuteroporphyrin when K⁺ (> 10 mM) is present (results not shown).

Effect of other cations

Na⁺ and Li⁺ decrease the uptake of deuterio-

Table 4. *Effect of cations on the efflux of Co-deuteroporphyrin from isolated rat liver mitochondria*

Mitochondria (about 2 mg of protein/ml) were incubated as described in the Materials and methods section. Incubation was for 60 min. The amount of Co-deuteroporphyrin synthesized was similar in all experiments [715 pmol/mg of protein (range 648–728)]. The results shown are the means and the ranges (in parentheses) for the separate experiments.

Addition	(mM)	Co-deuteroporphyrin released to the supernatant (%)
None	—	30 (25–34)
+K ⁺	10	14 (9–20)
	20	10 (7.5–14)
	100	6.5 (5–7.5)
+Na ⁺	10	30 (19–35)
	20	22 (16–28)
	100	7.5 (6–8)
+Li ⁺	10	24 (18–28)
	20	20 (8–22)
	100	5 (4–7)
+Ca ²⁺	0.5	15 (12.5–18.0)
	2.5	11.5 (10–13)
	5	6.5 (5–8)
+Mg ²⁺	0.5	12.5 (10–14)
	2.5	7.5 (6–8)
	5	3.3 (3–3.5)

porphyrin (Table 1) and the rate of synthesis of Co-deuteroporphyrin (Table 3); however, they do so less efficiently than K⁺.

Comparative results on the effect of K⁺, Na⁺, Li⁺, Ca²⁺ and Mg²⁺ on the release of Co-deuteroporphyrin are given in Table 4. After 60 min incubation the amount of Co-deuteroporphyrin synthesized is essentially the same whether or not cations are added. The amount of Co-deuteroporphyrin released to the medium, however, is markedly decreased when cations are added. In terms both of concentration and of changes associated with the cations, the inhibitory effect on the efflux decreases in the order: Mg²⁺ > Ca²⁺ > K⁺ > Li⁺ > Na⁺.

Efflux of Co-protoporphyrin

In the experiments so far described, deuteroporphyrin has been used as the porphyrin substrate.

There is, however, substantial evidence that deuteroporphyrin behaves as a substrate essentially like protoporphyrin (Jones & Jones, 1969, 1970; Barrett & Jones, 1978; Koller *et al.*, 1976; Koller & Romslo, 1980).

As shown in Fig. 7 Co-protoporphyrin escapes from the mitochondria by mechanisms qualitatively similar to those for Co-deuteroporphyrin. Thus, in a K⁺-depleted medium, the time–progress curve increases in a sigmoidal fashion, K⁺ (100 mM) abolishes the increased efflux that coincides with

Fig. 7. Time-progress curves for the release of Co-protoporphyrin from isolated rat liver mitochondria. Experimental conditions were as described in the Materials and methods section. ○, Control experiment in a K^+ -depleted medium; ■, with $4\mu M$ -globin; ●, with 100 mM-KCl; and ▲, with $4\mu M$ -globin and 100 mM-KCl.

uncoupling, and globin ($4\mu M$) mobilizes about 70 and 40% of the amount of Co-protoporphyrin synthesized in the absence and presence of 100 mM- K^+ respectively.

Discussion

The rate of efflux of metalloporphyrin from rat liver mitochondria increases with loose coupling and with the presence of metalloporphyrin-binding ligands in the incubation medium (Husby & Romslo, 1980; Husby *et al.*, 1980). As shown in the present experiments, the mobilization of metalloporphyrin is also markedly influenced by the ionic composition of the medium. K^+ decreases the efflux and thereby establishes a high concentration gradient of metalloporphyrin from mitochondria to the medium. The effect of K^+ is abolished only in part by high concentration of globin and it affects the release of Co-protoporphyrin in a way similar to its effect on Co-deuteroporphyrin release.

A high endogenous concentration of K^+ is essential to retain oxidative phosphorylation (Papa *et al.*, 1969; Gomez-Puyou *et al.*, 1970). However, it is unlikely that the effects of K^+ as reported here are through preservation of the integrity of the mitochondria, because: (1) the proton permeability and the respiratory-control ratio are essentially similar in a K^+ -depleted and in a K^+ -enriched medium (Fig. 4 and Opit & Charnock, 1965); (2) K^+ decreases the efflux of metalloporphyrin in tightly coupled mitochondria and in uncoupled mitochondria irrespective of whether or not metalloporphyrin-binding ligands are present in the incubation medium (Figs. 3 and 7, and Table 2); (3)

Ca^{2+} , which functions as a loose-coupler at the concentration used (Carafoli, 1979), decreases the efflux essentially as K^+ (Table 4); and (4) if the efflux were by simple diffusion after membrane disruption, the percentage efflux at 60 min should be much higher (Fig. 4).

K^+ inhibits the energy-dependent uptake of porphyrins by the mitochondria (Koller & Romslo, 1980, and Table 1) and thereby decreases the rate of metalloporphyrin synthesis (Fig. 6). However, during 60 min incubation, all the porphyrins added are chelated to cobalt (Fig. 6 and Table 4), but the amount of metalloporphyrin of the supernatant is still very low (Figs. 3, 4 and 7, and Table 4). Thus the release of metalloporphyrins is more susceptible to K^+ than is the uptake of porphyrins. Cations are well known to enhance the retention of respiratory substrates (Meisner *et al.*, 1972) and ADP/ATP (Meisner, 1971). The effect was originally ascribed to an increase in the positive charge density on the surface of the mitochondria (Meisner *et al.*, 1972). However, more recent studies have shown that the inhibitory effect of bivalent cations, at least on the ADP/ATP translocase, results from the formation of cation-nucleotide complexes that are not substrates for the translocase (Krämer, 1980; Brandolin *et al.*, 1980). The effect of the cations as seen in the present study can not be through replacement of Co^{2+} and the formation of cation-porphyrin complexes. More likely, the cation increases the ionic strength of the incubation medium and thereby favours the retention of lipophilic metalloporphyrins within the hydrophobic mitochondrial membrane. However, the effect of the cations can not be explained only through an increase in ionic strength; Mg^{2+} is a much more potent inhibitor of the efflux than are any of the univalent cations. Apparently Mg^{2+} (and to a lesser extent Ca^{2+}) has additional effects on the structure and function of the mitochondrial inner membrane (Ligeti & Horvath, 1980). For the uptake of substrate anions (Meisner *et al.*, 1972) and the translocation of ADP (Meisner, 1971) the inhibitory effect of the cations decreased in the order $Mg^{2+} > Ca^{2+} > K^+ > Li^+ > Na^+$, i.e. exactly in the same order as we find for the retention of metalloporphyrin. A challenging but speculative hypothesis would be that the influx of porphyrin is coupled to the efflux of metalloporphyrin through the operation of a carrier, and that the loading and reloading of the carrier is regulated in part by the energy state of the mitochondria and in part by the ionic strength and the presence of suitable acceptors of the incubation medium (Israels *et al.*, 1975; Husby & Romslo, 1980). By analogy to the ADP/ATP translocator, a porphyrin/haem translocator seems justifiable, at least in the hepatocyte, where more than 80% of the haem synthesized intramitochondrially is transported outside for mic-

rosomal cytochrome synthesis (Granick & Beale, 1978).

The effect of K^+ can only in part be counterbalanced by globin (Table 2). The effect cannot be on the association constant for the binding of metalloporphyrin to globin, because a high ionic strength has been shown to increase the association of metalloporphyrin to globin (Blauer & Silfen, 1978). Rather the effect of the cations may result from a spatial separation of the metalloporphyrin from globin. This could occur within the inner membrane or at the interphase between the inner membrane and the intermembrane space.

With regard to the biological significance of these types of experiments, it is noteworthy that Coprotoporphyrin behaves qualitatively essentially as does Co-deuteroporphyrin. However, since the rate of Co-protoporphyrin synthesis is below that of Co-deuteroporphyrin (Koller & Romslo, 1980), it follows that the gradient from the mitochondria to the incubation medium with Co-protoporphyrin is lower than when Co-deuteroporphyrin is present, i.e. mitochondria more readily release Coprotoporphyrin than Co-deuteroporphyrin.

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References

- Barrett, J. & Jones, O. T. G. (1978) *Biochem. J.* **174**, 277–281
- Blauer, G. & Silfen, J. (1978) in *Transport by Proteins* (Blauer, G. & Sund, H., eds.), p. 355, Walter de Gruyter, Berlin and New York
- Brandolin, G., Doussiere, J., Gulik, A., Gulik-Krzywicki, T., Lauquin, G. J. M. & Vignais, P. V. (1980) *Biochim. Biophys. Acta* **592**, 595–614
- Carafoli, E. (1979) *FEBS Lett.* **104**, 1–5
- Flatmark, T. & Romslo, I. (1975) *J. Biol. Chem.* **250**, 6433–6438
- Flatmark, T., Terland, O. & Helle, K. B. (1971) *Biochim. Biophys. Acta* **226**, 9–19
- Gomez-Puyou, A., Sandoval, F., Chavez, E. & Tuena, M. (1970) *J. Biol. Chem.* **245**, 5239–5247
- Granick, S. & Beale, S. I. (1978) *Adv. Enzymol.* **41**, 33–201
- Husby, P. & Romslo, I. (1980) *Biochem. J.* **188**, 459–465
- Husby, P., Müller-Eberhard, U. & Romslo, I. (1980) *Biochem. Biophys. Res. Commun.* **94**, 1345–1352
- Israels, L. G., Yoda, B. & Schachter, B. A. (1975) *Ann. N.Y. Acad. Sci.* **244**, 651–661
- 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
- Koller, M.-E. & Romslo, I. (1978) *Biochim. Biophys. Acta* **503**, 238–250
- Koller, M.-E. & Romslo, I. (1980) *Biochem. J.* **188**, 329–335
- Koller, M.-E., Romslo, I. & Flatmark, T. (1976) *Biochim. Biophys. Acta* **449**, 480–490
- Krämer, R. (1980) *Biochim. Biophys. Acta* **592**, 615–620
- Liget, E. & Horvath, L. I. (1980) *Biochim. Biophys. Acta* **600**, 150–156
- Meisner, H. (1971) *Biochemistry* **10**, 3485–3490
- Meisner, H., Palmieri, F. & Quagliariello, E. (1972) *Biochemistry* **11**, 949–954
- Müller-Eberhard, U. (1978) in *Transport by Proteins* (Blauer, G. & Sund, H. eds.), Walter de Gruyter, Berlin and New York
- Müller-Eberhard, U. & Morgan, W. T. (1975) *Ann. N.Y. Acad. Sci.* **244**, 624
- Opit, L. J. & Charnock, J. S. (1965) *Biochim. Biophys. Acta* **110**, 9–16
- Papa, S., Tager, J. M., Guerrieri, F. & Quagliariello, E. (1969) **172**, 184–186
- 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
- Sano, S. & Granick, S. (1961) *J. Biol. Chem.* **236**, 1173–1180
- Taketani, S. & Tokunaga, R. (1980) *Biochem. Biophys. Res. Commun.* **92**, 1343–1347
- Yoda, B. & Israels, L. G. (1972) *Can. J. Biochem.* **50**, 633–637