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PROTECTIVE EFFECT OF NUPERCAINE ON MITOCHONDRIAL STRUCTURE

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SUMMARY

A local anesthetic, nupercaine, incubated with rat liver mitochondria in sucrose medium reduces the damage to mitochondrial structure as observed in electron microscope.

INTRODUCTION

Mitochondrial membranes contain phospholipase A_2 (refs 1, 2) which catalyses the breakdown of phospholipid to yield the corresponding lyso-derivatives. It has been shown that this enzyme is located primarily in the outer membrane of mitochondria[3,4] although the presence of phospholipase A_2 in the inner membrane cannot be excluded[4]. Recent studies have indicated that extraction of the mitochondria with ammoniacal acetone solubilises the enzyme[5]. Waite *et al.*[6] suggested that phospholipase A_2 plays an important role in the control of mitochondrial permeability and swelling. On the other hand, Seppala *et al.*[7] and Scherphof *et al.*[8] showed that local anesthetics inhibit both venom phospholipase activity and mitochondrial phospholipase A_2. Scarpa and Lindsay[9] showed that a local anesthetic, nupercaine, was effective in both stabilizing energy-linked function of isolated mitochondria and maintaining the integrity of mitochondrial phospholipids. It was also found[10] that mitochondria incubated with phospholipase C took on the appearance of submitochondrial particles packed inside mitochondrial membranes. The question arises whether nupercaine would protect the structure of mitochondria against the damage caused by endogenous mitochondrial phospholipase A_2. To answer this question is the aim of the present work.

MATERIALS AND METHODS

Rat liver mitochondria were prepared as described previously[11], in Ca^{2+}-free 0.25 M sucrose–10 mM Tris–HCl (pH 7.4). Isolated mitochondria were suspended in the same solution at a final protein concentration of 50 mg/ml. Protein was estimated by the biuret method[12].

Mitochondria (5 mg protein) were incubated at 30 °C in a medium which contained: 250 mM sucrose (Ca^{2+} free), 10 mM Tris–HCl (pH 7.4) and, in some

cases, 500 μM nupercaine (Ciba A.G.). The total volume was 1 ml. After incubation the samples were centrifuged for 40 s at 15000 × *g*, and the pellets obtained were fixed in 2% OsO_4 for 1 h. Procedures used for electron microscopy were as described by Popinigis *et al.*[13]. Specimens were examined in a JEM 7A electron microscope at 80 kV.

RESULTS

The addition of nupercaine to the freshly prepared mitochondria (Fig. 1A) caused only a non-significant increase in the volume of the inner compartment, and a narrowing of the intercristal space and of the outer compartment (Fig. 1B). After 15 min of incubation at 30 °C, the control mitochondria were round (Fig. 2A), their outer compartment and intercristal space was reduced in volume, and the matrix had a lower electron density. After 15 min of incubation in the presence of nupercaine (Fig. 2B), the mitochondria looked the same as they did at the beginning of the experiment. Following longer periods of incubation (30–60 min) the structural signs of swelling of the control mitochondria increased (Figs 3A and 4A). The mitochondria were larger, their matrix was electron transparent, the cristae mitochondriales were short and angular and, in the final stage of these experiments (Fig. 4A), almost absent. Many mitochondria lost the outer lamina of the mitochondrial membrane. At the same time mitochondria incubated with nupercaine (Figs 3B and 4B) remained as large as they were after 15 min of incubation but had very narrow cristae mitochondriales, with a granular matrix of moderate electron density, and an almost collapsed outer compartment.

DISCUSSION

The electron microscopic observations presented in this paper show that, during incubation of mitochondrial suspension in sucrose medium, damage of mitochondrial structure takes place and increases with time of incubation. Morphological changes are similar to these which were observed during incubation of mitochondrial suspension with oleate[14] or phospholipase C[15]. In the experimental conditions applied, nupercaine reduced swelling of mitochondria. Scarpa and Lindsay[9] have shown that during storage of rat liver mitochondria at 0–4 °C, the respiratory control ratio decreases progressively together with an increase in the conversion of mitochondrial phosphatidylethanolamine and phosphatidylcholine to their corresponding lysoderivatives. They have also shown that nupercaine was effective both in preventing the decrease of respiratory control ratio and in maintaining the integrity of mitochondrial phospholipids[9]. The ultrastructural changes of rat liver mitochondria presented above are probably caused by the action of phospholipase A_2. Under our conditions, mitochondria were prepared in sucrose–Tris–HCl medium (without EDTA); Thiers and Vallee[16] showed that, after a careful isolation by the sucrose method, rat liver mitochondria contain about 10 nmoles Ca^{2+} per mg protein, which is probably sufficient for the activation of phospholipase A_2. The protective effect of nupercaine would suggest that degradation of mitochondrial phospholipids by endogenous phospholipase A_2 is responsible for the damage of mitochondrial structure. The damage of mitochondrial structure, observed in electron micrographs (Figs 2A–4A), are probably caused by: (a) swelling of mitochondria produced by free fatty acids

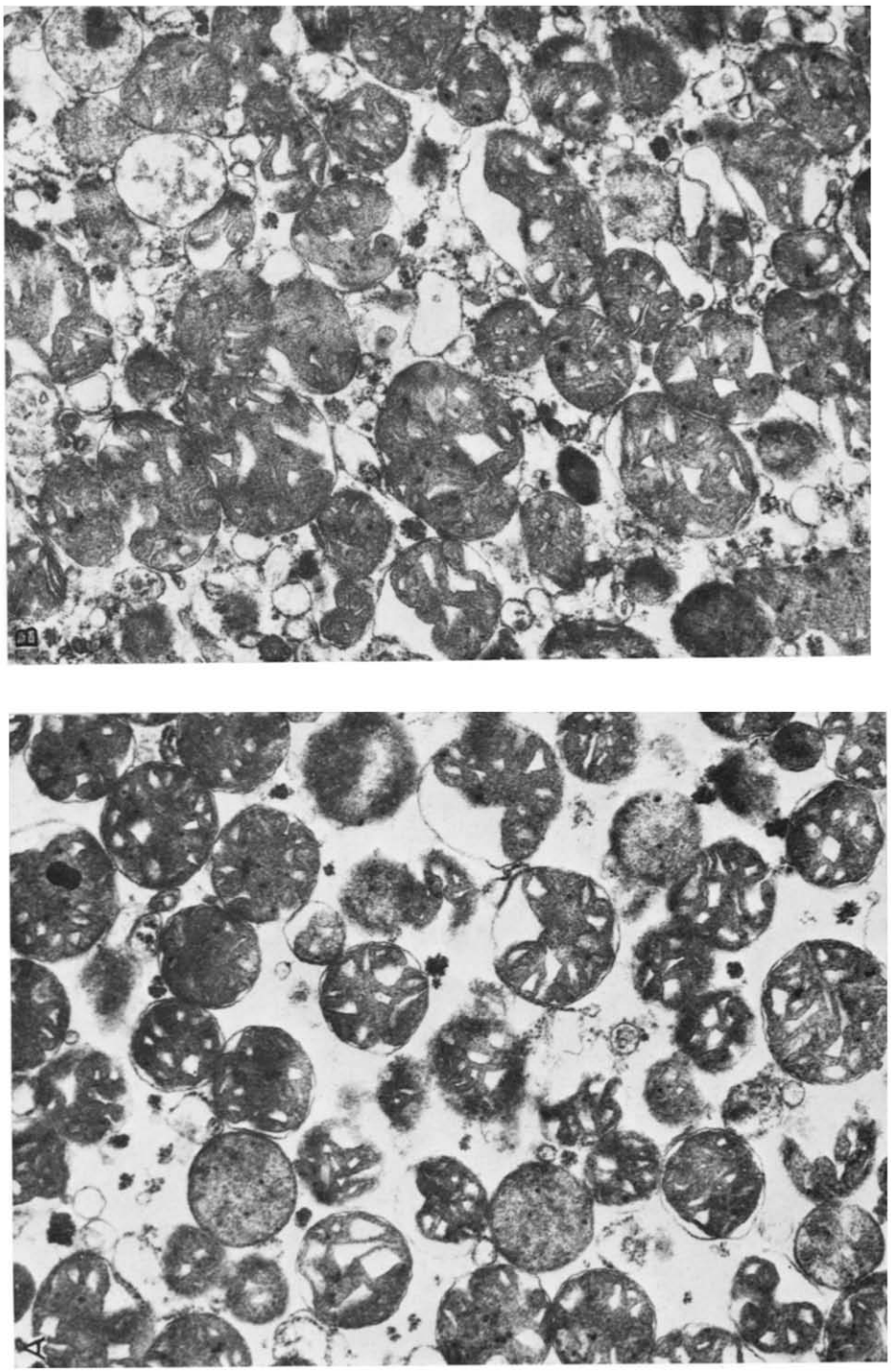

Fig. 1. Freshly isolated mitochondria suspended in 250 mM sucrose–10 mM Tris–HCl, without (A) and with 0.5 mM nupercaine (B). Experimental conditions as described in the text. Mitochondrial pellets were prepared at zero time. ×22000.

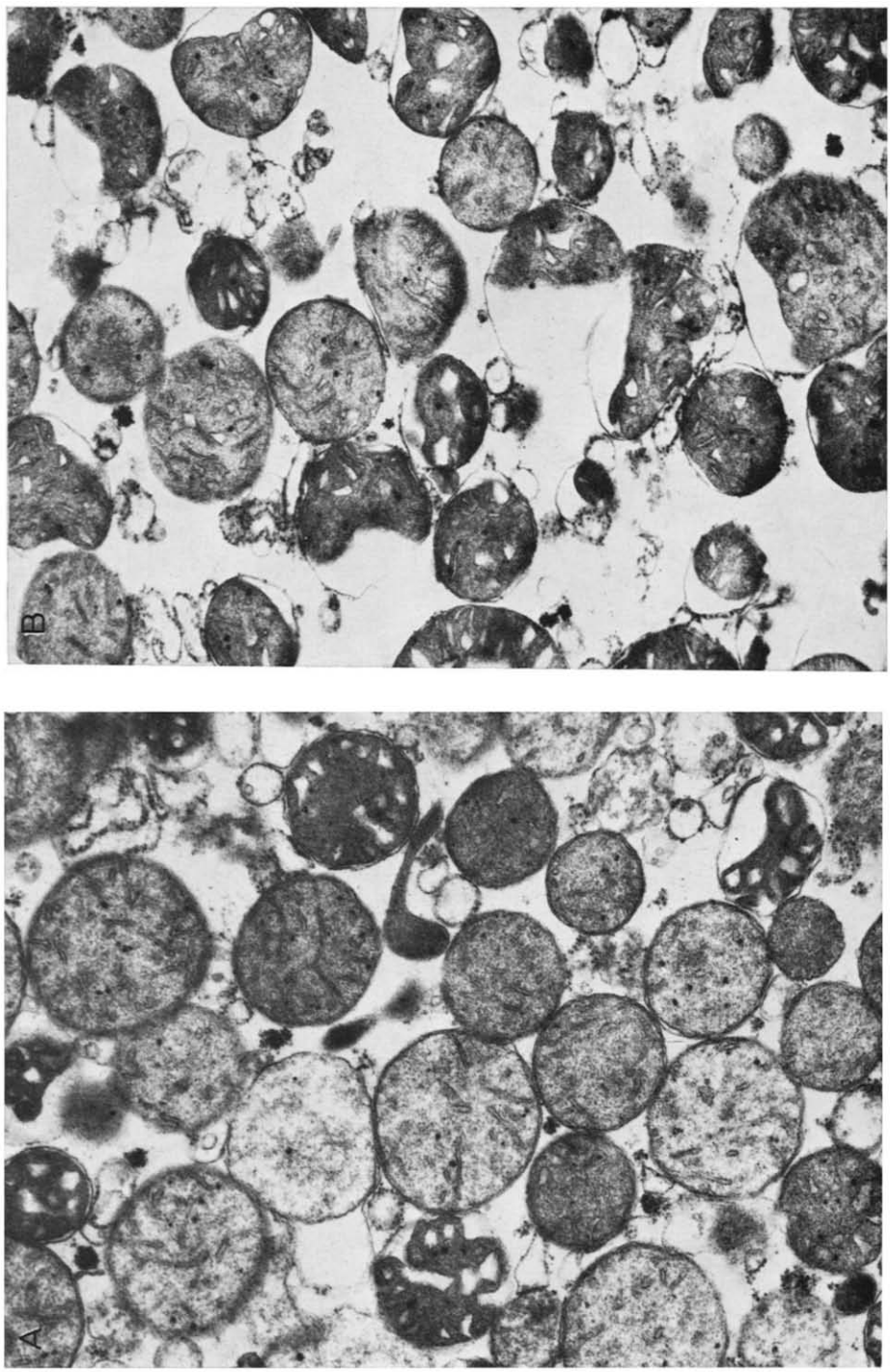

Fig. 2. Ultrastructural changes of mitochondria after 15 min of incubation without (A) and with 0.5 mM nupercaine (B). Experimental conditions as described in the text. ×22000.

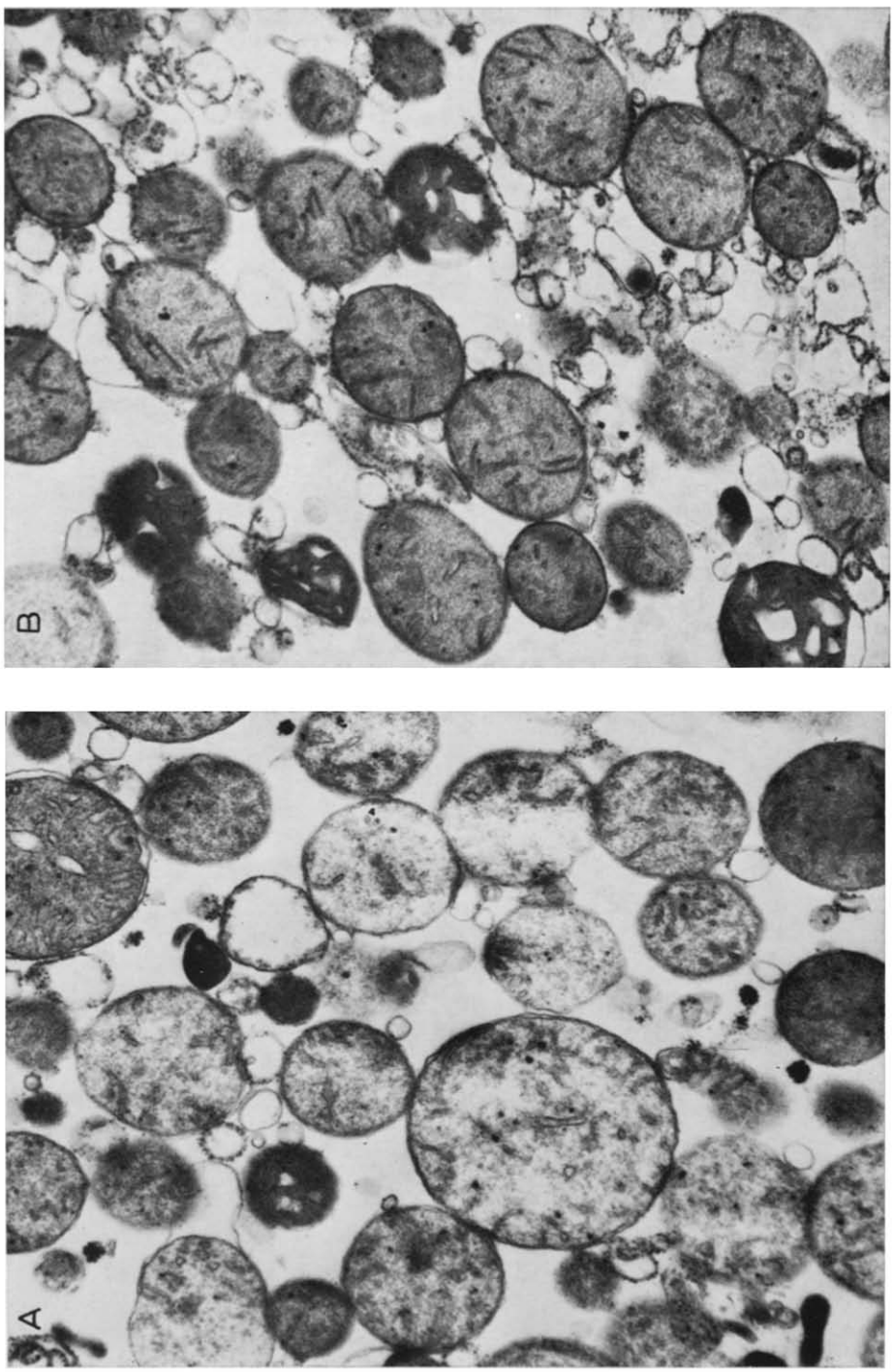

Fig. 3. Ultrastructural changes of mitochondria after 30 min of incubation without (A) and with 0.5 mM nupercaine (B). Experimental conditions as described in the text. × 22000.

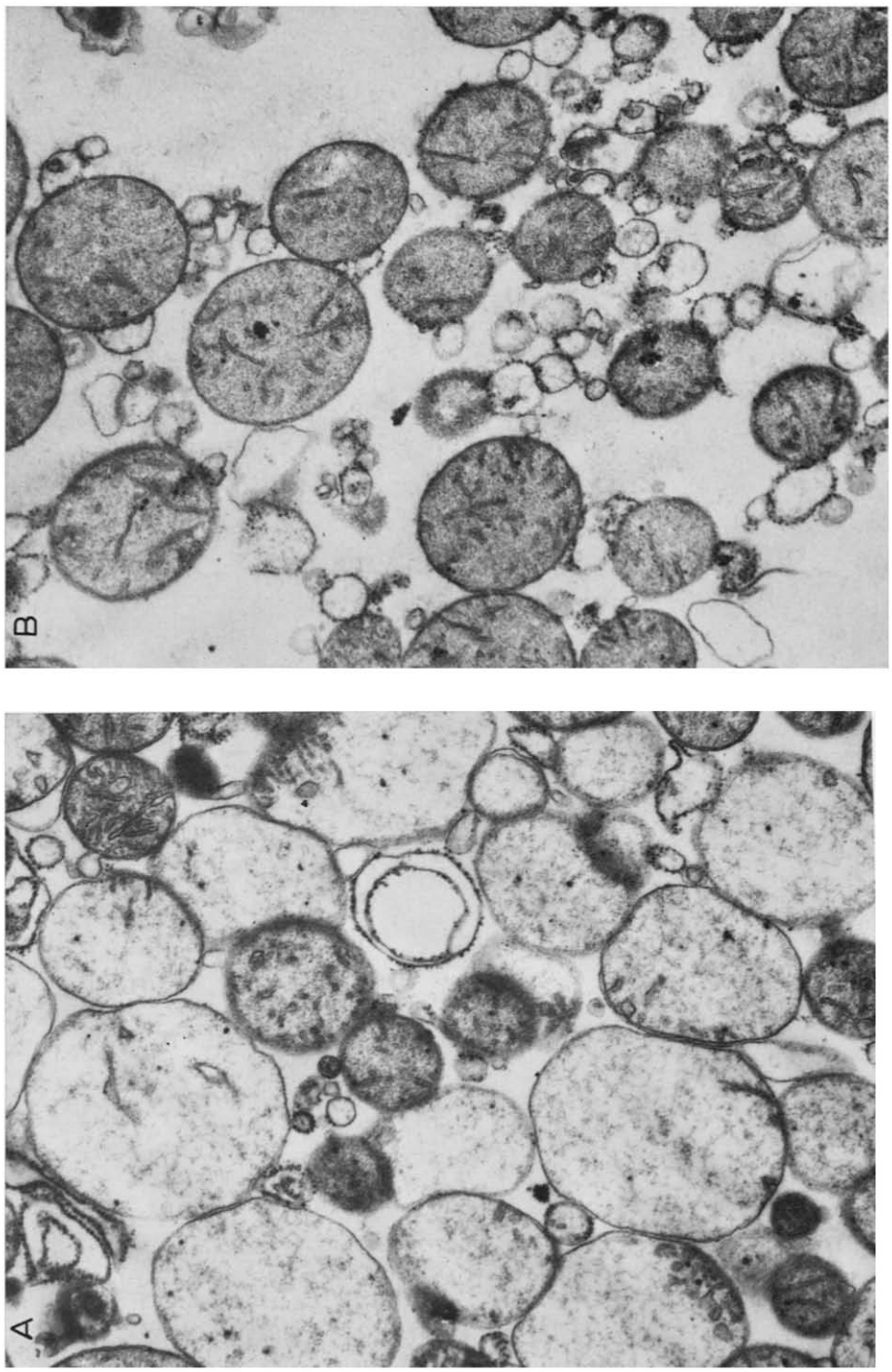

Fig. 4. Ultrastructural changes of mitochondria after 60 min of incubation without (A) and with 0.5 mM nupercaine (B). Experimental conditions as described in the text. ×22000.

liberated from membranes, as suggested by Wojtczak and Lehninger[17]; (b) cleavage of the phospholipid constituents of the mitochondrial membrane.

The most characteristic structural feature observed with nupercaine is its action on the mitochondrial membrane. Ultrastructural changes observed after the addition of this local anesthetic to freshly prepared mitochondria (Fig. 1B) are probably produced by the interaction of this compound with the mitochondrial membrane. The interactions of local anesthetics with membrane phospholipids has been recently reviewed[18]. Feinstein[19] proposed a complex formation between phosphatidylserine molecules and tetracaine. According to him the polar aromatic nitrogen and positively charged tertiary alkyl nitrogen groups of tetracaine would orientate toward the negatively charged ionised phosphate groups of two phosphatidylserine molecules. Acidic phospholipids such as phosphatidylserine, phosphatidylinositol and phosphatidylethanolamine are present both in the outer and inner mitochondrial membrane[20]. We assume that nupercaine, like tetracaine, forms complexes with acidic phospholipids of the mitochondrial membrane. According to Scarpa and Lindsay[9], the protective effect of nupercaine on structural integrity is due to the direct action of this anesthetic on phospholipase A_2 activity. It is possible that, on top of a direct action on phospholipase A_2, nupercaine might inhibit the action of this enzyme by forming nupercaine–phospholipids complexes in the mitochondrial membrane. These complexes would be converted to corresponding lyso-derivatives only very slowly. However it remains as yet a matter of speculation whether this mechanism plays an important role in the induced damage of mitochondrial structure.

Our electron microscopical observations strongly support the suggestion of Scarpa and Lindsay[9] that the activity of endogenous phospholipase A_2 is largely responsible for the damage to mitochondria structure which results in a loss of energy-linked functions; the addition of nupercaine during incubation was shown to maintain the integrity of mitochondrial structure for a long period of time.

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REFERENCES

1 Scherphof, G. L. and Van Deenen, L. L. M. (1965) *Biochim. Biophys. Acta* 98, 204–206
2 Scherphof, G. L., Waite, M. and Van Deenen, L. L. M. (1966) *Biochim. Biophys. Acta* 125, 406–409
3 Nachbaur, J. and Vignais, P. M. (1968) *Biochem. Biophys. Res. Commun.* 33, 315–320
4 Waite, M. (1969) *Biochemistry* 8, 2536–2542
5 Waite, M. and Sisson, P. (1971) *Biochemistry* 10, 2377–2383
6 Waite, M., Van Deenen, L. L. M., Ruigrok, T. J. C. and Elbers, P. F. (1969) *J. Lipid Res.* 10, 599–608
7 Seppala, A. J., Saris, N. E. L. and Gaufin, M. L. (1971) *Biochem. Pharmacol.* 20, 305–313
8 Scherphof, G. L., Scarpa, A. and Toorenenbergen, A. (1972) *Biochim. Biophys. Acta* 270, 226–240
9 Scarpa, A. and Lindsay, J. G. (1972) *Eur. J. Biochem.* 27, 401–407
10 Burstein, C., Loyter, A. and Racker, E. (1971) *J. Biol. Chem.* 246, 4075–4082
11 Aleksandrowicz, Z., Świerczyński, J. and Żelewski, L. (1972) *Eur. J. Biochem.* 31, 300–307

12 Layne, E. (1957) in *Methods of Enzymology* (Colowick, S. P. and Kaplan, N. O., eds), Vol. 3, pp. 447–454, Academic Press, New York
13 Popinigis, J., Wrołkowa, Teresa and Świerczyński, J. (1971) *Biochim. Biophys. Acta* 245, 70–79
14 Wojtczak, L., Bogucka, K., Sarzała, M. G. and Żaluska, H. (1969) *FEBS Symp.* 17, 79–92
15 French, S. W., Todoroff, T., Norum, M. L. and Thrig, T. J. (1972) *Exp. Mol. Pathol.* 16, 16–32
16 Thiers, R. E. and Vallee, B. (1957) *J. Biol. Chem.* 226, 911–920
17 Wojtczak, L. and Lehninger, A. L. (1961) *Biochim. Biophys. Acta* 51, 442–456
18 Papahadjopoulos, D. (1972) *Biochim. Biophys. Acta* 265, 169–186
19 Feinstein, M. B. (1964) *J. Gen. Physiol.* 48, 357–374
20 Levy, M., Toury, R. and Sauner, M. T. (1969) *FEBS Symp.* 17, 33–42