Functional changes in rat liver mitochondria on administration of 2-methyl-4-dimethylaminoazobenzene

P. SAIKUMAR and C. K. RAMAKRISHNA KURUP

Department of Biochemistry, Indian Institute of Science, Bangalore 560012, India

(Received 18 June 1984/Accepted 28 August 1984)

1. Administration of 2-methyl-4-dimethylaminobenzene in the diet (0.1%, w/w) for 85–90 days doubled the content of mitochondria in the livers of rats. The azodye was covalently bound to liver proteins, and about 15% of the amount found in liver was associated with the mitochondrial fraction. 2. Mitochondria isolated from the livers of azodye-fed animals showed drastically lowered ability to oxidize NAD⁺-linked substrates. The inhibited electron-transfer step was the reduction of ubiquinone. The organelles showed a large increase in succinate oxidase activity. 3. The activity of cytochrome oxidase and the content of cytochrome *aa*₃ were substantially higher in these organelles. 4. Azodye-fed animals showed depressed serum cholesterol concentrations. The content of ubiquinone in liver also registered a small increase.

The potential health hazard in the indiscriminate use of azodyes as colour additives in the cosmetic, pharmaceutical and food industries was spotlighted by the discovery by Miller & Miller (1953) that certain methyl-substituted aminoazobenzenes are very potent hepatocarcinogens. In contrast with the carcinogenic azodyes that decreased hepatic mitochondria, the weakly carcinogenic isomer 2-methyl-4-dimethylaminoazobenzene (2-Me-DAB) increased the population of the organelles in the livers of experimental animals (Price *et al.*, 1949; Potter *et al.*, 1950; Wu *et al.*, 1973).

It was reported by us recently (Saikumar & Kurup, 1984*a,b*) that 2-Me-DAB is a potent inhibitor of electron transport and coupled phosphorylation *in vitro*. The susceptible reaction step was identified as the NADH-linked reduction of ubiquinone. It was decided to test whether the inhibitory action of the azodye was exerted *in vivo*. We have investigated in fair detail the effect of administration of the azodye to the rat on liver mitochondria, with special emphasis on quantity and functional efficiency. The results are presented and their implications discussed in the present paper.

Materials and methods

Male albino rats (140–160 g) obtained from the Central Animal Facility of this Institute were fed

Abbreviation used: 2-Me-DAB, 2-methyl-4-dimethylaminoazobenzene.

on a commercial (Hind Lever) diet and were used in the experiments reported here. Experimental animals were administered with the azodye in the powdered diet (0.1%, w/w) for 85–90 days.

The method for the preparation of mitochondria has been described previously (Kurup *et al.*, 1970). Subcellular fractionation of liver homogenate (10%, w/v) in 0.25 M-sucrose was done by differential centrifugation (Krishnakantha & Kurup, 1972). Pure fractions of nuclei were prepared by the method of Wang (1967). Submitochondrial particles were prepared by the method of Graven *et al.* (1967).

Polarographic determination of oxidative phosphorylation was made in a Gilson model K-ICT-C Oxygraph. The reaction system contained (final concentrations) 50 mM-Tris/HCl buffer, pH 7.4, 20 mM-potassium phosphate buffer, pH 7.4, 2 mM-EDTA, 7 mM-MgCl₂, 50 mM-sucrose and 1 mg (protein) of freshly isolated mitochondria in a total volume of 1.4 ml. After the addition of the substrate (2 mM-glutamate + 4 mM-malate or 17 mM-succinate) the rate of State 3 oxidation (Chance & Williams, 1955) was measured by the addition of 200 μ M-ADP. Succinate oxidase and NADH oxidase activities of submitochondrial particles were also determined by polarography, the reaction medium being essentially the same as described above. Cytochrome oxidase activity was determined by the oxidation of ferrocytochrome *c* (Smith & Conrad, 1956). The activity of NADH dehydrogenase was determined with K₃Fe(CN)₆ as electron acceptor (King & Howard, 1967).

Succinate dehydrogenase was assayed with phenazine methosulphate and 2,6-dichlorophenol-indophenol (King, 1967).

Protein-bound 2-Me-DAB was determined essentially as described by Chauveau & Decloitre (1968). The subcellular fraction (50–100 mg of protein) was treated successively with 10% (w/v) HClO_4 , 5% HClO_4 , ethanol, ethanol/diethyl ether (3:1, v/v) at 80°C and acetone. The dry powder was dissolved in 85% (w/v) formic acid, and the absorption spectrum of the solution was recorded against an equal amount (protein) of a similarly treated sample from control animals. The amount of protein-bound azodye was calculated from the absorption spectrum by using the equation (Chauveau & Decloitre, 1968):

$$\text{Azodye } (\mu\text{g/ml}) = 10.2[A_{520} - \frac{1}{2}(A_{480} + A_{560})]$$

The contents of cytochromes aa_3 , b and c were determined from difference spectra (reduced – oxidized), $\text{Na}_2\text{S}_2\text{O}_4$ and ascorbate + $\text{NNN}'\text{N}'$ -tetramethyl- p -phenylenediamine being used for reduction (Gazzotti *et al.*, 1980).

The content of cholesterol in serum was determined after saponification and extraction into light petroleum (b.p. 40–60°C; Stadtman, 1957). Hepatic ubiquinone was estimated by the decrease in A_{275} on reduction with NaBH_4 (Joshi *et al.*, 1963).

Mitochondrial counts were made by the erythrocyte dilution method (Baranowicz, 1972). Protein was determined by the biuret method (Gornall *et al.*, 1949), deoxycholate being used for solubilization. All solutions were prepared in water double-distilled in an all-quartz apparatus. Solutions were adjusted to pH 7.4 before use. Sucrose solutions were prepared fresh daily.

Results

Mitochondrial population

Rats administered with the azodye (0.1%, w/w) in the diet for 90 days showed a consistent decrease in body weight (by 20%) and an increase in liver weight (by 10%). Consequently, as observed previously (Allard *et al.*, 1957; Wu *et al.*, 1973), the liver-weight/body-weight ratio increased by 30% in these animals (results not shown). In agreement with the well-documented mitochondria-proliferating property of the compound (Price *et al.*, 1949, 1950; Potter *et al.*, 1950; Allard *et al.*, 1957; Lafontain & Allard, 1964; Wu *et al.*, 1973), administration of the azodye resulted in a substantial increase (by 75%) in the protein recovered in the mitochondrial fraction (Table 1). Mitochondrial counts also registered a similar increase ($5.7 \times 10^{11} \pm 1.2 \times 10^{11}$ and $3.8 \times 10^{11} \pm 0.9 \times 10^{11}$ per g of fresh liver in the dye-fed and control

Table 1. *Effect of administration of 2-Me-DAB to the rat on the protein content of hepatic subcellular fractions* Animals were fed with the azodye (0.1%, w/w) in the diet for 85–90 days. The values are the means $\pm$ S.D. for 12 independent determinations. The values in parentheses represent the percentages of protein recovered in the fractions, taking the total homogenate protein (212 ± 33 mg for control and 208 ± 35 mg for azodye-fed animals respectively) to be 100.

Subcellular fraction	Protein in fractions (mg/g of fresh liver)	
	Control	2-Me-DAB-fed
Nuclei	40 ± 5 (19)	39 ± 5 (17)
Mitochondria	37 ± 5 (18)	65 ± 7 (28)
Microsomal fraction	45 ± 7 (22)	48 ± 6 (21)
Cytosol	84 ± 2 (41)	77 ± 9 (34)

Table 2. *Distribution of protein-bound 2-Me-DAB in the subcellular fractions of rat liver*

Rats were fed with 2-Me-DAB (0.1%, w/w) in the diet for 85–90 days. Protein-bound azodye was determined as described in the Materials and methods section. The values represent the means $\pm$ S.D. for eight to ten animals. The values in parentheses represent the percentage recoveries of the azodye in the fractions, taking the amount in whole liver to be 100.

Hepatic fraction	Concentration of 2-Me-DAB	
	(pmol/mg of protein)	(nmol/g of fresh liver)
Whole liver	111 ± 13	22.8 ± 3.3
Nuclei	41 ± 12	1.6 ± 0.5 (8)
Mitochondria	45 ± 6	3.0 ± 0.4 (15)
Microsomal fraction	106 ± 13	6.0 ± 2.0 (29)
Cytosol	141 ± 22	9.9 ± 1.5 (48)

animals respectively). Neither the total liver protein nor the protein recovered in the other subcellular fractions showed any significant change (Table 1).

Protein-bound azodye

It is known that about 40% of the ingested azodye is not metabolized and gets covalently bound to the cellular macromolecules, particularly to hepatic proteins (Miller & Miller, 1953). The pattern of distribution of the protein-bound dye presented in Table 2 reveals that about 75% of the dye remained bound to cytosolic and microsomal proteins. The mitochondrial fraction accounted for 15% of the total amount present in the liver. The pattern is in general agreement with earlier reports (Price *et al.*, 1950; Chauveau *et al.*, 1974). It may also be pointed out that considerable quantities of the azodye was bound to serum proteins (15.5 ± 1.6 pmol/mg of protein), the circulating concentration being 878 ± 12.5 nM.

Table 3. *Effect of administration of 2-Me-DAB to the rat on oxidative phosphorylation by liver mitochondria*
Animals were fed with 2-Me-DAB (0.1%, w/w) in the diet for 85–90 days. O₂ uptake was measured by polarography. The rates of State 3 oxidation (ADP present; Chance & Williams, 1955) are expressed in ng-atoms of O/min per mg of protein. Respiratory control index was calculated as the ratio of the rates of O₂ uptake in State 3 to that in State 4 (ADP exhausted). The values are the means $\pm$ S.D. for six independent determinations (animals). Statistical significance (control versus experimental): * P < 0.05; ** P < 0.01.

Substrate	Activity	Control	2-Me-DAB-fed
Glutamate + malate	State 3 oxidation	123 $\pm$ 12	71 $\pm$ 14**
	Respiratory control index	9.1 $\pm$ 0.1	5.8 $\pm$ 0.7
	ADP/O	2.6 $\pm$ 0.04	2.5 $\pm$ 0.1
Succinate	State 3 oxidation	213 $\pm$ 27	240 $\pm$ 23
	Respiratory control index	5.6 $\pm$ 0.6	6.4 $\pm$ 0.6*
	ADP/O	1.7 $\pm$ 0.1	1.7 $\pm$ 0.2

Table 4. *Effect of administration of 2-Me-DAB to the rat on the oxidative activities of hepatic submitochondrial particles*
Animals were fed with the azodye (0.1%, w/w) in the diet for 85–90 days. The particles were prepared by ultrasonic disruption of liver mitochondria. The values are the means $\pm$ S.D. for six independent determinations (animals). Statistical significance (control versus experimental): * P < 0.05; ** P < 0.01.

Activity	Control	2-Me-DAB-fed
NADH oxidase (ng-atoms of O/min per mg of particle protein)	625 $\pm$ 135	294 $\pm$ 33**
Succinate oxidase (ng-atoms of O/min per mg of protein)	399 $\pm$ 74	601 $\pm$ 29*
Cytochrome oxidase (nmol of ferrocytochrome <i>c</i> oxidized/min per mg of protein)	1025 $\pm$ 191	1626 $\pm$ 277**
NADH dehydrogenase [nmol of K ₃ Fe(CN) ₆ reduced/min per mg of protein]	5825 $\pm$ 497	4987 $\pm$ 220*
Succinate dehydrogenase (nmol of dichlorophenol-indophenol reduced/min per mg of protein)	692 $\pm$ 42	1060 $\pm$ 116**

Oxidative phosphorylation

In view of the observation (Saikumar & Kurup, 1984a,b) that the azodye was a potent inhibitor of NADH-linked electron transport, liver mitochondria isolated from 2-Me-DAB-fed animals were tested for their functional efficiency. The data presented in Table 3 reveal that active oxidation of NAD⁺-linked substrates was drastically inhibited in these organelles. It should be mentioned that the decrease in respiratory control reflects only the inhibition of State 3 oxidation. There was no stimulation of State 4 oxidation (ADP-exhausted; Chance & Williams, 1955). Succinate oxidation showed a consistent increase (Table 3). A decrease in NAD⁺-linked oxidation in hepatic mitochondria of azodye-fed animals had been reported previously (Arcos *et al.*, 1969; Wu *et al.*, 1973).

The differential effects exerted by the azodye on NADH-linked and succinate oxidations were seen more strikingly in submitochondrial particles. The NADH oxidase activity was significantly depressed (by 53%) on dye administration. In contrast, the dehydrogenase activity showed only a marginal decrease (Table 4). Interestingly, both succinate oxidase and cytochrome oxidase activities registered substantial increases (50–60%) in the treated animals (Table 4).

We have shown earlier (Saikumar & Kurup, 1984a) that *in vitro* the azodye inhibited the

reduction of cytochrome *b* and also the reduction of ubiquinone. The results presented in Fig. 1 clearly indicate that the rate of reduction of cytochrome *b* in particles prepared by ultrasonication from the livers of 2-Me-DAB-fed animals was drastically decreased when NADH was used as electron donor (Expt. 2). The inhibited rate of electron transport is further reflected in the increase by more than 100% in the time for the onset of anaerobiosis after the addition of NADH. In contrast, when succinate was used as electron donor, neither the time for anaerobiosis nor the rate of reduction of cytochrome *b* showed any perceptible difference between control and experimental samples (Fig. 1, Expt. 4). In the experimental samples, which show enhanced succinate oxidase activity, a shortening of the time for the onset of anaerobiosis was expected. It may be noted that no preincubation of the particles with succinate was made in these experiments.

Because of the inherent difficulty in the direct measurement of the reduction of endogenous ubiquinone (Kroger & Klingenberg, 1967), an indirect approach was used. The reduction of neotetrazolium [3,3'-(4,4-biphenylene)-2,2',5,5'-tetraphenylditetrazolium chloride] to the formazan is stimulated by exogenously added ubiquinone (Lester & Smith, 1961; Aithal & Ramasarma, 1971). Also, exogenous ubiquinone is known to accept electrons from its endogenous counterpart (Rama-

Fig. 1. Effect of administration of 2-Me-DAB to the rat on the rate of reduction of cytochrome *b* in hepatic submitochondrial particles

The reaction system (1 ml) contained 1 mg of protein of submitochondrial particles from control (Expts. 1 and 3) or 2-Me-DAB-fed (for 85–90 days) rats (Expts. 2 and 4). Substrate (NADH in Expts. 1 and 2 and succinate (Succ) in Expts. 3 and 4) was added as indicated, and the reduction of cytochrome *b* was measured at 562 nm–577 nm (Saikumar & Kurup, 1984a). The time for the onset of anaerobiosis and reduction of cytochrome *b* is indicated. The spectrum was recorded with an Aminco DW2a dual-wavelength spectrophotometer.

sarma & Lester, 1960). Ubiquinone-stimulated neotetrazolium reduction is totally inhibited by rotenone when NADH is the electron donor (Saikumar & Kurup, 1984a). Therefore the stimulated rate of neotetrazolium reduction on addition of exogenous ubiquinone is a measure of the rate of reduction of endogenous ubiquinone.

Submitochondrial particles obtained from the livers of azodye-fed animals showed a distinct inability to stimulate neotetrazolium reduction on addition of ubiquinone when NADH was used as the electron donor, but not when succinate was used as the substrate (Table 5).

Content of cytochromes

The results in Table 4 showed that in azodye-fed animals succinate oxidase and cytochrome oxidase activities were increased. To test whether the increase was due to an increase in the concentration of electron-transport components, the concentrations of cytochromes in mitochondria were determined from difference spectra. The results presented in Table 6 reveal that administration of the azodye led to a significant increase in the content of cytochrome *aa*₃. The fact that the increase was also observed in submitochondrial particles rules out the possibility that the increase was an artifact resulting from the loss of proteins from the organelles, particularly from the matrix.

Table 5. Effect of administration of 2-Me-DAB to the rat on the rate of reduction of neotetrazolium by hepatic submitochondrial particles

The azodye (0.1%, w/w) was fed in the diet for 90 days. The reduction of neotetrazolium was determined by A_{510} (Susheela & Ramasarma, 1973). Ubiquinone-9 (0.5 mM), this being the major lipid quinone in the rat (Ramasarma, 1961), was added as emulsion in water. The values are the means for four independent determinations (animals). Statistical significance (no addition versus ubiquinone addition): * $P < 0.01$.

Substrate	Addition	Neotetrazolium reduction (nmol of formazan formed/min per mg of particle protein)	
		Control	2-Me-DAB-fed
NADH	None	328 ± 65	289 ± 84
	Ubiquinone	654 ± 34*	385 ± 26
Succinate	None	67 ± 37	75 ± 50
	Ubiquinone	851 ± 368*	799 ± 302*

Both cytochrome *b* and cytochrome *c* also showed marginal increases.

Serum cholesterol

It is well recognized that drugs such as clofibrate exert their antihypercholesterolaemic effects

Table 6. *Effect of administration of 2-Me-DAB to the rat on the concentration of cytochromes in hepatic mitochondria*

The azodye (0.1%, w/w) was fed in the diet for 85–90 days. The content of cytochromes was calculated from difference (reduced – oxidized) spectra as described in the Materials and methods section. The ϵ values used for calculation were 16 (605 nm–630 nm), 20 (560 nm–575 nm) and $19.1 \text{ mm}^{-1} \cdot \text{cm}^{-1}$ (550 nm–540 nm) for cytochromes *a*, *b* and *c* respectively. The values are the means $\pm$ S.D. for six independent determinations (animals). Statistical significance (control versus experimental): * $P < 0.05$; ** $P < 0.01$.

Preparation	Cytochrome	Concentration (pmol/mg of protein)	
		Control	2-Me-DAB-fed
Mitochondria	<i>aa</i> ₃	122 $\pm$ 2	205 $\pm$ 18**
	<i>b</i>	187 $\pm$ 10	198 $\pm$ 14
	<i>c</i> + <i>c</i> ₁	273 $\pm$ 11	311 $\pm$ 30*
Submitochondrial particles	<i>aa</i> ₃	343 $\pm$ 38	525 $\pm$ 85**
	<i>b</i>	379 $\pm$ 64	432 $\pm$ 53
	<i>c</i> + <i>c</i> ₁	532 $\pm$ 113	639 $\pm$ 118

Table 7. *Effect of administration of 2-Me-DAB to the rat on the concentration of serum cholesterol and hepatic ubiquinone*

Animals were fed with 2-Me-DAB (0.1%, w/w) in the diet for 90 days. The values are the means $\pm$ S.D. for five independent determinations. Statistical significance (control versus experimental): * $P < 0.05$; ** $P < 0.01$.

	Control	2-Me-DAB-fed
Serum cholesterol (mg/100 ml)	67 $\pm$ 6	48 $\pm$ 2**
Hepatic ubiquinone (nmol/g fresh wt.)	174 $\pm$ 32	277 $\pm$ 21*

through the proliferation of liver mitochondria, which possess an enzyme for the cleavage of the side chain of the lipid (Kurup *et al.*, 1970; Krishnakantha & Kurup, 1972; Gear *et al.*, 1974; Lee & Whitehouse, 1963). It was decided to test whether proliferation of mitochondria by the azodye also produced a similar effect. The results presented in Table 7 reveal that in the treated animals the concentration of cholesterol in the circulation decreased substantially (by 30%).

Even though ubiquinone in the liver is distributed in all the subcellular fractions (Sastry *et al.*, 1961; Jayaraman & Ramasarma, 1963), more than 75% of it is accounted for by the mitochondrial fraction. Proliferation of mitochondria could therefore lead to an increase in the concentration of the quinone in liver. The results in Table 7 show that azodye administration caused only a modest increase (25%) in the content of ubiquinone in liver.

Discussion

The observation that significant quantities of the azodye remained bound to proteins and could

be determined by spectral methods applicable to the unchanged molecule strengthens the view that about 40% of the ingested dye remains in the system covalently bound to macromolecules (Miller & Miller, 1953; Warwick, 1967; Chauveau & Decloitre, 1968; Chauveau *et al.*, 1974). The point of interest is that 15% of the azodye present in the liver is associated with the mitochondrial fraction. The fact that the native azodye is bound to inner-membrane proteins explains not only the impaired activity of NADH-linked oxidation in these mitochondria but also the close agreement in the site of inhibition observed *in vitro* and *in vivo*. The lack, due to covalent bonding, of mobility of the compound in the lipid milieu of the membrane accounts for the inability of the azodye to exert its uncoupler-like action observed *in vitro* (Saikumar & Kurup, 1984a,b). One could speculate that the dye might be bound preferentially to the Complex I segment of the respiratory chain.

The increase in the activities of the succinate oxidase system in the mitochondria of azodye-fed animals may be a compensatory mechanism evolved to circumvent the loss of activity in Complex I segment of the electron-transport chain. In fact, the proliferation of mitochondria itself could reflect this mechanism.

Another point that merits discussion is the physiological side effect of the proliferation of hepatic mitochondria. It is becoming increasingly accepted that the hypolipidaemic function of serum-cholesterol-depressing drugs such as clofibrate rests to a great extent on their organelle-proliferating potential. The enhancement in the population of mitochondria enables more rapid clearance of cholesterol by oxidation (Gear *et al.*, 1974). The observation that 2-Me-DAB also depresses the serum cholesterol concentration lends additional support to this view.

Even though both clofibrate and 2-Me-DAB

proliferate mitochondria and depress the serum cholesterol concentration, many dissimilarities may be noted in their action *in vivo*. Thus the administration of clofibrate leads to drastic increases (2–3-fold) in the hepatic ubiquinone content (Krishnakantha & Kurup, 1974), whereas the increase observed with 2-Me-DAB is much smaller (25%). Similarly, in contrast with the large increases in the activities of mitochondrial carnitine acetyltransferase and α -glycerophosphate dehydrogenase observed in clofibrate-treated animals (Krishnakantha & Kurup, 1972; Mittal & Kurup, 1980), administration of the azodye had little effect on the activities of these enzymes (P. Saikumar & C. K. R. Kurup, unpublished work). A third and more significant difference is that, whereas administration of clofibrate leads to a marginal (10–15%) decrease in the oxidative activity of hepatic mitochondria (Kurup *et al.*, 1970; Krishnakantha *et al.*, 1975), no specific part of the respiratory chain is inhibited as is observed on administration of the azodye.

Financial assistance from the University Grants Commission, New Delhi, is acknowledged.

References

- Aithal, H. N. & Ramasarma, T. (1971) *Biochem. J.* **123**, 677–682
- Allard, C., De Lamirande, G. & Cantero, A. (1957) *Cancer Res.* **17**, 862–879
- Arcos, J. C., Mattie, J. T., Gosch, H. H. & Fabian, J. A. (1969) *Cancer Res.* **29**, 1298–1306
- Baranowicz, J. W. (1972) *Anal. Biochem.* **45**, 1–5
- Chance, B. & Williams, G. R. (1955) *J. Biol. Chem.* **217**, 409–427
- Chauveau, J. & Decloitre, F. (1968) *Int. J. Cancer* **3**, 88–95
- Chauveau, J., Meunier, N. & Benoit, A. (1974) *Int. J. Cancer* **13**, 1–8
- Gazzotti, P., Malmstrom, K. & Crompton, M. (1980) in *Membrane Biochemistry* (Carafoli, E. & Semenza, G., eds.), pp. 62–76, Springer-Verlag, Berlin
- Gear, A. R. L., Albert, A. D. & Bednarek, J. M. (1974) *J. Biol. Chem.* **249**, 6495–6504
- Gornall, A. G., Bardawill, G. J. & David, M. M. (1949) *J. Biol. Chem.* **177**, 751–766
- Graven, S. N., Lardy, H. A. & Estrada-O, S. (1967) *Biochemistry* **6**, 365–371
- Jayaraman, J. & Ramasarma, T. (1963) *Arch. Biochem. Biophys.* **103**, 258–266
- Joshi, V. C., Jayaraman, T. & Ramasarma, T. (1963) *Indian J. Exp. Biol.* **1**, 113–123
- King, T. E. (1967) *Methods Enzymol.* **10**, 322–331
- King, T. E. & Howard, R. L. (1967) *Methods Enzymol.* **10**, 275–294
- Krishnakantha, T. P. & Kurup, C. K. R. (1972) *Biochem. J.* **130**, 167–175
- Krishnakantha, T. P. & Kurup, C. K. R. (1974) *Indian J. Biochem. Biophys.* **11**, 51–53
- Krishnakantha, T. P., Mohan, V. P., Chhabra, S. & Kurup, C. K. R. (1975) *Indian J. Biochem. Biophys.* **12**, 31–34
- Kroger, A. & Klingenberg, M. (1967) *Curr. Top. Bioenerg.* **2**, 151–193
- Kurup, C. K. R., Aithal, H. N. & Ramasarma, T. (1970) *Biochem. J.* **116**, 773–779
- Lafontain, J. G. & Allard, C. (1964) *J. Cell Biol.* **22**, 143–172
- Lee, M. J. & Whitehouse, M. W. (1963) *Biochem. J.* **89**, 189–195
- Lester, R. L. & Smith, A. L. (1961) *Biochim. Biophys. Acta* **47**, 475–496
- Miller, J. A. & Miller, E. C. (1953) *Adv. Cancer Res.* **1**, 340–396
- Mittal, B. & Kurup, C. K. R. (1980) *Biochim. Biophys. Acta* **619**, 90–97
- Potter, V. R., Price, J. M., Miller, E. C. & Miller, J. A. (1950) *Cancer Res.* **10**, 28–35
- Price, J. M., Miller, E. C., Miller, J. A. & Weber, G. M. (1949) *Cancer Res.* **9**, 398–402
- Price, J. N., Miller, E. C., Miller, J. A. & Weber, G. M. (1950) *Cancer Res.* **10**, 18–27
- Ramasarma, T. (1961) *J. Sci. Ind. Res. Sect. A* **20**, 492–512
- Ramasarma, T. & Lester, R. L. (1960) *J. Biol. Chem.* **235**, 3309–3314
- Saikumar, P. & Kurup, C. K. R. (1984a) *Biochim. Biophys. Acta* **766**, 263–266
- Saikumar, P. & Kurup, C. K. R. (1984b) *Indian J. Biochem. Biophys.* in the press
- Sastry, P. S., Jayaraman, J. & Ramasarma, T. (1961) *Nature (London)* **189**, 577
- Smith, L. & Conrad, S. (1956) *Arch. Biochem. Biophys.* **63**, 403–413
- Stadtman, T. C. (1957) *Methods Enzymol.* **3**, 392–393
- Susheela, L. & Ramasarma, T. (1973) *Biochim. Biophys. Acta* **292**, 50–63
- Wang, T. Y. (1967) *Methods Enzymol.* **12**, 417–421
- Warwick, G. P. (1967) *Eur. J. Cancer* **3**, 227–233
- Wu, B. C., Argus, M. F. & Arcos, J. C. (1973) *Chem.-Biol. Interact.* **7**, 399–416