Decreased Activities of Ubiquinol:Ferricytochrome c Oxidoreductase (Complex III) and Ferrocytochrome c:Oxygen Oxidoreductase (Complex IV) in Liver Mitochondria from Rats with Hydroxycobalamin[c-lactam]-induced Methylmalonic Aciduria*

(Received for publication, March 15, 1991)

Stephan Krahnenbuhl‡, Mei Chang, Eric P. Brass, and Charles L. Hoppel$  
From the Departments of Medicine and Pharmacology, Case Western Reserve University, Veterans Administration Medical Center, Cleveland, Ohio 44106

Rats treated with hydroxycobalamin[c-lactam] (HCCL), a cobalamin analogue that induces methylmalonic aciduria, have increased hepatic mitochondrial content and increased oxidative metabolism of pyruvate and palmitate per hepatocyte. The present studies were undertaken to characterize oxidative metabolism in isolated liver mitochondria from rats treated with HCCL. After 5–6 weeks, state 3 oxidation rates for diverse substrates are reduced in mitochondria from HCCL-treated rats. Similar reductions of mitochondrial oxidation rates are obtained with dinitrophenol-uncoupled mitochondria excluding defective phosphorylation as a cause for the observed decrease in mitochondrial oxidation. The activities of mitochondrial oxidases are reduced in HCCL-treated rats and demonstrate a defect in complex IV. Investigation of the complexes of the respiratory chain reveals a 52% decrease of ubiquinol:ferricytochrome c oxidoreductase (complex III) activity and a 72% decrease of ferrocytochrome c:oxygen oxidoreductase (complex IV) activity in mitochondria from 5–6-week HCCL-treated rats as compared with controls. Liver mitochondria from HCCL-treated rats also demonstrate decreased cytochrome content per mg of mitochondrial protein (25% decrease of cytochrome b and 52% decrease of cytochrome a + a₃ as compared with control rats). The HCCL-treated rat represents an animal model for the study of the consequences of respiratory chain defects in liver mitochondria.

Vitamin B₁₂ deficiency decreases the activity of the two cobalamin-dependent enzymes L-methylmalonyl-CoA mutase and methionine synthetase in humans (1) and in experimental animals (2, 3). L-Methylmalonyl-CoA mutase catalyzes the conversion of L-methylmalonyl-CoA to succinyl-CoA which is the rate-limiting step in cellular propionate utilization (4). In rats, vitamin B₁₂ deficiency resulting in decreased activity of L-methylmalonyl-CoA mutase can be achieved by feeding a vitamin B₁₂-free diet (2, 3). Administration of the vitamin B₁₂ analogue hydroxycoobalaminc[c-lactam] (HCCL) is an alternative method to produce decreased activity of L-methylmalonyl-CoA mutase (5). Surprisingly, rats treated with HCCL not only showed metabolic consequences of L-methylmalonyl-CoA mutase inhibition (hepatic accumulation of abnormal acyl-CoAs and methylmalonic aciduria) (6, 7) but also had increased oxidative metabolism of pyruvate and palmitate per hepatocyte (7, 8), which was explained by increased hepatocellular mitochondrial content (8). In contrast to the increased oxidative metabolism of palmitate and pyruvate per hepatocyte, state 3 oxidation rates in isolated liver mitochondria from HCCL-treated rats (expressed per mg of mitochondrial protein) for pyruvate or palmitoylcarnitine as substrates were decreased (8), suggesting a defect in oxidative metabolism of liver mitochondria.

The aim of the present investigation was to characterize the function and to define any enzymatic defect in the electron transport chain of isolated liver mitochondria from HCCL-treated rats. The results of the current studies show that HCCL treatment over 5–6 weeks leads to decreased activities of ubiquinol:ferricytochrome c oxidoreductase (complex III) and ferrocytochrome c:oxygen oxidoreductase (complex IV) of the electron transport chain of liver mitochondria and is associated with decreased mitochondrial cytochrome b and cytochrome a + a₃ contents.

MATERIALS AND METHODS

Animals—Male Fischer 344 rats (Charles River Laboratories, Portage, MI) were used for all experiments. Two to three rats were housed per cage with free access to drinking water and normal rat chow (Lab Chows, Purina Mills Inc., St. Louis, MO). After an acclimation period of 10 days, an osmotic mini-pump (Alza Corp., Palo Alto, CA, model 2002) containing either saline (control animals) or hydroxycoobalaminc[c-lactam] (HCCL-treated animals) was implanted subcutaneously under ether anesthesia. Hydroxycoobalaminc[c-lactam] was delivered at a rate of 2 μg/h. In animals treated for 5–6 weeks, a second mini-pump (content identical to the first mini-pump) was placed 3 weeks after the first pump implantation. At the time point of the first mini-pump implantation, the animals weighed 250 ± 20 g (n = 24). In agreement with previous studies (5–7), both body weights and liver weights were not different between HCCL-treated and control rats after 2–3 weeks and after 5–6 weeks of treatment with HCCL (data not shown).

Mitochondrial Isolation and Oxidative Metabolism—Liver mitochondria were isolated as described by Hoppel et al. (9). Briefly, fed...
rats were killed by decapitation between 08:00 and 09:00 a.m. The liver was quickly removed and placed in ice-cold MSB buffer (290 mM mannitol, 70 mM sucrose, 5 mM MOPS, pH 7.4). The liver was rinsed, blotted, weighed, minced, and washed with cold MSB buffer. A 10% suspension (w/v) of the minced liver containing 2 mM EDTA buffer was prepared using a Potter-Elvehjem homogenizer with a loose fitting tefzel stirrer and cell deliverer. The homogenate was stored at 700 °C for 10 min, and mitochondria were isolated by centrifugation of the supernatant at 7000 x g for 10 min. The resulting mitochondrial pellet was washed twice with MSB buffer and finally diluted to contain approximately 50 mg of mitochondrial protein per ml.

Oxygen consumption by intact mitochondria was measured in a chamber equipped with a Clark-type oxygen electrode (Yellow Springs Instrument Co.) at 30 °C. The incubations contained 1 mg/ml mitochondrial protein in 80 mM KCl, 50 mM MOPS, 1 mM EGTA, 5 mM KH2PO4, pH 7.4. Defatted bovine serum albumin (1 mg/ml; w/v) was added to the incubations containing fatty acids as substrates. The final volume of the incubations was 500 µl for incubations containing fatty acids as substrate and 1000 µl for all other substrates. After depletion of endogenous substrates by the addition of ADP, the substrate was added to the incubation, and state 3 respiration was initiated by addition of ADP (final concentration, 100 µM ADP/ml). State 3 and state 4 respiration were determined and calculated according to Chance (10) as ADP-stimulated and ADP-limited respiration, respectively. Respiratory control ratio (ratio of state 3 to state 4 respiration) were calculated according to Elsabrok (11).

Enzyme activities were determined with freeze-thawed mitochondria as described by Blair et al. (12) and were performed at 37 °C using the oxygen electrode. Incubations contained 29 mM potassium phosphate (pH 7.4), 0.1 mM EDTA, 0.15 mM oxidized cytochrome c, mitochondrial protein, and substrates (added last) in a final volume of 500 µl. Activities were determined in the presence of specific inhibitors and were calculated as the difference of uninhibited minus inhibited rates. NADH oxidation activity was measured with 2.8 mM NADH as substrate, with and without 7.5 µM rotenone as inhibitor. Succinate oxidation was measured in the presence of 0.8 mM duraquinone and 40 mM succinate, with and without 10 µM of antimycin A as inhibitor. Duroquinol oxidase was measured with 2 mM duroquinol as substrate, with and without 10 µM of antimycin A as inhibitor. Cytochrome c oxidase was measured in the presence of 0.24 mM N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD) and 7.2 mM ascorbate, with and without 2 mM sodium azide as inhibitor.

Enzyme Activities and Cytochrome Content—Enzyme activities were measured at 30 °C using solubilized mitochondria or liver homogenate (1 mg of mitochondrial protein or 200 µl of 10% liver homogenate (w/v) solubilized in 0.1 M potassium phosphate buffer containing 0.1% bovine serum albumin (w/v), 3 mM sodium azide, 100 µM DCIP, and 50 mM potassium phosphate, pH 7.4, in a final volume of 1 ml. After an equilibration period of 5 min, reactions were started by the addition of 20 mM succinate, and the decrease in absorbance at 600 nm was monitored. Activities were calculated using an extinction coefficient of 12.8 absorbance units per millimole per cm for DCIP (17). The activity of succinate cytochrome c oxidoreductase (control and FCCP-treated) was measured in the presence of 10 µM rotenone and 0.25 mM MPTD (18). The activities of the mitochondrial electron transport chain were determined spectrophotometrically. Activity was determined spectrophotometrically. Activity was determined spectrophotometrically.

Succinate-2,6-dichlorophenol (DCIP) oxidoreductase (complex II) activities were determined spectrophotometrically. Activities contained 100 µg of mitochondrial protein, 0.1 mM EDTA, 0.1% bovine serum albumin (w/v), 3 mM sodium azide, 100 µM DCIP, and 50 mM potassium phosphate, pH 7.4, in a final volume of 1 ml. After an equilibration period of 5 min, reactions were started by the addition of 20 mM succinate, and the decrease in absorbance at 600 nm was monitored. Activities were calculated using an extinction coefficient of 12.8 absorbance units per millimole per cm for DCIP (17). The activity of succinate cytochrome c oxidoreductase (control and FCCP-treated) was measured in the presence of 10 µM rotenone and 0.25 mM MPTD (18). The activities of the mitochondrial electron transport chain were determined spectrophotometrically. Activity was determined spectrophotometrically. Activity was determined spectrophotometrically.
in state 3 oxidation rates was approximately 30% for pyruvate, L-glutamate, and fatty acids (requiring complexes I, III, and IV of the electron transport chain), 38% for succinate (requiring complexes II, III, and IV), 47% for duroquinol (requiring complexes III and IV), and 51% for TMPD/L-ascorbate (requiring complex IV). State 4 oxygen consumption was not different between mitochondria isolated from control and HCCL-treated rats; thus, the respiratory control ratios showed the same decline as the state 3 oxidation rates (data not shown). When oxidative phosphorylation was uncoupled with dinitrophenol, state 3 oxidation rates were reduced by 36% for L-glutamate and by approximately 50% for duroquinol and TMPD/L-ascorbate as substrates (Table II), excluding phosphorylation defects as a reason for the observed reduction in state 3 oxygen consumption.

Activities of mitochondrial oxidases (measured polarographically) were investigated to help localize the affected site and to exclude changes in the composition of the inner mitochondrial membrane and/or transport defects of substrates as causes for the observed decrease in state 3 oxidation rates with liver mitochondria from HCCL-treated rats. Consistent with the state 3 oxidation rates obtained with intact mitochondria, oxidase activities were reduced in mitochondrial preparations from HCCL-treated rats as compared with preparations from control rats (Table III). The reductions in oxidase activity with mitochondrial preparations from HCCL-treated as compared with control rats were 60.2% for NADH oxidase (requiring complexes I, III, and IV), 47.5% for succinate oxidase (requiring complexes II, III, and IV), 69.9% for duroquinol oxidase (requiring complexes III and IV), and 57.4% for cytochrome c oxidase (requiring complex IV).

The results obtained by the polarographic methods demonstrated a defect at complex IV in liver mitochondria from HCCL-treated rats; however, additional defects in the other complexes of the electron transport chain could not be excluded. Therefore, activities of discrete components of the electron transport chain were determined. Consistent with the polarographic studies, the defect at complex IV was confirmed spectrophotometrically (Table IV). The first order rate constant of cytochrome c oxidase expressed per mg of mitochondrial protein was decreased by 72% in mitochondrial preparations from HCCL-treated rats as compared with control rats. Activities of rotenone-sensitive NADH:ferricytochrome c oxidoreductase (requiring complexes I and III) and succinate:ferricytochrome c oxidoreductase (requiring complexes II and III) were reduced by 76 and 42%, respectively, in mitochondrial preparations from HCCL-treated as compared with control rats. In contrast, rotenone-insensitive NADH:ferricytochrome oxidoreductase activity, which requires NADH:cytochrome b oxidoreductase (in the outer mitochondrial membrane [16], was not affected by HCCL treatment. Similarly, activities of complex I (NADH:ferricyanide oxidoreductase and NADH:dioxyquinone oxidoreductase) and of complex II (succinate dehydrogenase and succinate:DCIP oxidoreductase) were not different between mitochondria from HCCL-treated and control rats (Table IV). The suspected defect at complex III was confirmed directly by the 32% reduction in state 3 oxygen consumption.

Table I

	

| Complexes | Control | HCCL-treated |
|-----------|---------|--------------|
| Complexes I, III, IV | 40.5 ± 5.5 (6) | 49.2 ± 5.0 (3) |
| 20 mM 3-hydroxybutyrate | 49.2 ± 5.0 (3) | 40.6 ± 5.0 (3) |
| 20 mM pyruvate | 48.6 ± 3.5 (6) | 31.4 ± 4.9 (3)* |
| 20 mM L-glutamate | 82.0 ± 11.3 (12) | 81.3 ± 11.8 (3) |
| 0.8 mM octanoate | 54.7 ± 8.6 (6) | 49.5 ± 5.4 (3) |
| 0.16 mM palmitate | 4.0 ± 4.6 (6) | 39.5 ± 4.0 (3) |
| Complexes II, III, IV | 160 ± 25 (12) | 151 ± 26 (3) |
| Complexes III, IV | 160 ± 25 (12) | 151 ± 26 (3) |
| Complex IV | 49.8 ± 7.9 (7)** |
| 1 mM Duroquinol | 186 ± 29 (6) | ND |
| 7.2 mM L-Ascorbate + 0.24 mM TMPD | 182 ± 6 (6) | ND |

*Significant differences (p < 0.05) between HCCL-treated and control rats are indicated by *.
**Between the two groups of HCCL-treated rats by +. ND, not determined.
Electron Transport Chain Defects in HCCL-treated Rats

21001
decrease of the ubiquinol:ferricytochrome c oxidoreductase activity in mitochondrial preparations from HCCL-treated rats as compared with preparations from control rats (Table IV).

Reduced activities of complex III or complex IV of the electron transport chain are frequently associated with decreased contents of mitochondrial cytochrome b or cytochrome a + a3 (28-32). As compared with control rats, the content of cytochrome a + a3 (expressed per mg of mitochondrial protein) was decreased by 20% in 2-3 week HCCL-treated rats with no change in cytochrome b (Table V). After a treatment period of 5-6 weeks, the mitochondrial content of both cytochrome a + a3 and cytochrome b were decreased by 52 and by 25%, respectively, and the mitochondrial cytochrome c1 content was increased by 96% in HCCL-treated as compared with control rats.

**DISCUSSION**

The function of the respiratory chain was investigated in isolated liver mitochondria from rats treated with the vitamin B12 analogue HCCL. HCCL-treated rats showed decreased activities of ubiquinol:ferricytochrome c oxidoreductase (complex III) and ferrocytochrome c: oxygen oxidoreductase (complex IV) with decreased function of the electron transport chain after 5-6 weeks of HCCL treatment. These defects were associated with decreased mitochondrial content of cytochrome a + a3 and cytochrome b.

After a treatment period of 2-3 weeks, state 3 oxygen consumption by isolated mitochondria was not different between HCCL-treated and control rats. In contrast, after 5-6 weeks of HCCL treatment, state 3 oxidation rates for mitochondria from HCCL-treated rats were reduced for most substrates studied (Table I). The reduction in state 3 oxidation rates was greater for substrates with high oxidation rates ( duroquinol and TMPD/ascorbate) than for substrates showing lower oxidation rates ( pyruvate, L-glutamate, succinate, and fatty acids). In contrast to mitochondrial state 3 oxygen consumption, mitochondrial cytochrome a + a3 content was already reduced after 2-3 weeks of HCCL treatment, and a further reduction was observed after 5-6 weeks of HCCL treatment, combined with a reduction in mitochondrial cytochrome b content. The finding that the mitochondrial cytochrome a + a3 content can be reduced without impairment of the function of the electron transport chain is consistent with observations made in human electron transport chain defects (28-32) and suggests that complex IV is normally not rate-limiting for the activity of the electron transport chain. Since complex III is proposed to be rate-limiting for the activity of the electron transport chain in liver mitochondria (23), the observed decrease in oxidative metabolism of isolated mitochondria from 5-6 week HCCL-treated rats probably reflects decreased activity of complex III and not of complex IV.

**Table II**

*Oxygen consumption by uncoupled isolated rat liver mitochondria*

Uncoupling was performed by the addition of 0.1 mM dinitrophenol to the mitochondrial incubations. State 3 oxygen consumption was measured in a test chamber equipped with a Clark electrode at 30 °C. The number of incubations with individual mitochondrial preparations was 6 for both groups. Results are expressed as nanomoles/ min/mg of mitochondrial protein and are presented as mean ± S.D. Significant differences (p < 0.05) between control and HCCL-treated rats are indicated by *.

| Substrate Used | Control | HCCL-treated, 5-6 weeks |
|----------------|---------|-------------------------|
| 20 mM glutamate | 105 ± 17 | 67.2 ± 7.8* |
| 20 mM succinate | 138 ± 28 | 125 ± 19 |
| 1 mM duroquinone | 238 ± 46 | 110 ± 15* |
| 7.2 mM L-ascorbate + 0.24 mM TMPD | 180 ± 18 | 92.0 ± 13.1* |

**Table III**

*Activities of mitochondrial oxidases*

Activities of mitochondrial oxidases were measured with freeze-thawed mitochondria as described under "Materials and Methods." Oxygen consumption was monitored in a test chamber equipped with a Clark electrode at 30 °C. The number of incubations with individual mitochondrial preparations was 6 for both groups. Results as mean ± S.D. Significant differences (p < 0.05) between control and HCCL-treated rats are indicated by *.

| Activity assayed | Electron transport chain complexes required | Control | HCCL-treated, 5-6 weeks |
|-----------------|----------------------------------------------|---------|-------------------------|
| NADH oxidase    | I, II, III, IV                               | 299 ± 78 | 119 ± 23* |
| Succinate oxidase | II, III, IV                                  | 91.1 ± 36.3 | 48.0 ± 8.9* |
| Duroquinol oxidase | III, IV                                      | 511 ± 289 | 159 ± 136* |
| Cytochrome c oxidase | IV                                      | 861 ± 228 | 367 ± 216* |

**Table IV**

*Activities of subunits of the respiratory chain*

Cholate-solubilized mitochondria (1 mg of mitochondrial protein per ml of 100 mM phosphate buffer, pH 7.4, containing 1% cholate, w/v) were used for these measurements. Activities were determined spectrophotometrically as described under "Materials and Methods." The number of determinations with individual mitochondrial preparations was 6 for both groups. Results are presented as mean ± S.D. and are expressed as milliunits/mg of mitochondrial protein (except for ferrocytochrome c: oxygen oxidoreductase where the first order rate constant is given as 1/min/mg of mitochondrial protein). Significant differences between control and HCCL-treated rats are indicated by *.

| Activity assayed | Electron transport chain complexes required | Control | 5-6 week HCCL-treated |
|-----------------|----------------------------------------------|---------|-----------------------|
| Ferrocytochrome c: oxygen oxidoreductase | IV                                      | 115 ± 20 | 31.8 ± 10.8* |
| NADH:ferricytochrome c oxidoreductase | Rotenone insensitive | I, III | 205 ± 74 | 217 ± 46 |
|                  | Rotenone sensitive                           | II, III | 46.0 ± 21.1 | 11.1 ± 12.1* |
| NADH:ferricyanide oxidoreductase | II                                      | 56.2 ± 22.9 | 32.6 ± 11.2* |
| NADH:d duroquinone oxidoreductase | II                                      | 2150 ± 310 | 2360 ± 120 |
| Succinate dehydrogenase | II                                      | 35.2 ± 7.1 | 31.0 ± 8.8 |
| Succinate:DCIP oxidoreductase | No duroquinone added | II | 25.5 ± 5.0 | 27.2 ± 3.5 |
|                  | 500 µM duroquinone added                     | II | 16.1 ± 4.4 | 15.2 ± 3.6 |
| Ubiquinol:ferricytochrome c oxidoreductase | III | 53.8 ± 9.5 | 36.7 ± 5.2* |
Electron Transport Chain Defects in HCCL-treated Rats

Table V
Cytochrome content in isolated mitochondria

|                  | Control          | 2-3 weeks       | 5-6 weeks       |
|------------------|------------------|-----------------|-----------------|
|                  |                  | 20 ± 0.02 (3)*  | 12 ± 0.01 (9)*+ |
| Cytochrome a + a 0 | 0.25 ± 0.02 (12) |                 |                 |
| Cytochrome b      | 0.24 ± 0.02 (12) |                 |                 |
| Cytochrome c1     | 0.14 ± 0.01 (12) |                 |                 |
| Cytochrome c      | 0.19 ± 0.02 (12) |                 |                 |

Decreased mitochondrial ubinunone content could contribute to the observed decrease of the electron transport chain activity of isolated mitochondria from HCCL-treated rats. To approach this possibility, succinate:DCIP oxidoreductase activity (complex II) was determined both with and without addition of duroquinone (Table IV). Since DCIP accepts electrons directly from ubiquinol (34, 35), succinate:DCIP oxidoreductase activity measured without addition of exogenous ubiquinone is decreased in endogenous ubiquinone deficiency and can be normalized by the addition of exogenous ubiquinone (36). Succinate:DCIP oxidoreductase activities obtained in the current studies (no differences between HCCL-treated and control animals both in the absence and in the presence of 0.5 mM duroquinone, Table IV) are therefore consistent with a normal functional ubiquinone pool in mitochondria from HCCL-treated rats.

Consistent with a previous report in HCCL-treated rats (8), mitochondrial protein content per g of liver was increased in 5-6 week HCCL-treated as compared with control rats. Previous studies in HCCL-treated rats showed an increase in mitochondrial protein content as early as 2-3 weeks after the start of HCCL treatment (8), a time point where the function of the respiratory chain is unaltered (Table I). These findings suggest that increased mitochondrial content in livers from HCCL-treated rats is not a direct consequence of defects in the electron transport chain. It is possible that the metabolic abnormalities produced by the inhibition of l-methylmalonyl-CoA mutase by HCCL (hepatic accumulation of unusual acyl-CoAs and methylmalonic aciduria), which appear early after HCCL administration (7), are responsible for the mitochondrial proliferation in HCCL-treated rats. When mitochondrial biogenesis is accelerated, decreased mitochondrial cytochrome a + a 0 and/or cytochrome b content could result from an insufficient availability of the respective apoproteins and/or heme groups. In support of this hypothesis, hepatic mitochondrial proliferation in rats induced by hyperthyroidism is associated with decreased mitochondrial cytochrome b content (37). It is possible that the decrease in mitochondrial cytochrome a + a 0 and cytochrome b content in human mitochondrial disorders (28-32) is a consequence of mitochondrial proliferation which is frequently observed in human defects of the electron transport chain (30-32, 38).

In humans, decreased complex III activity is a recognized cause for mitochondrial myopathy, mitochondrial cardiomyopathy, and/or mitochondrial encephalomyopathy (28-32). A possible impairment of hepatic mitochondrial function has only been described in one of these patients (31). Similar to 5-6 week HCCL-treated rats (6), plasma glucose and ketone body concentrations were unaltered during starvation in this patient (31), ruling out severe defects in hepatic intermediary metabolism. In rats treated with HCCL over 5-6 weeks, compensatory mechanisms including mitochondrial prolifer-

Acknowledgments—We thank Dr. A. Zinn and Dr. L. Sayre for their helpful comments on the manuscript and L. Ruff, K. Patel, and J. Turkel for technical assistance.

REFERENCES
1. Rosenberg, L. E., and Fenton, W. A. (1989) The metabolic Basis of Inherited Disease (Scriver, C. R., Beaudet, A. L., Sly, W. S., and Valle, D., eds) pp. 821-844, McGraw-Hill, New York
2. Frenkel, E. P., and White, J. D. (1973) Lab. Invest. 29, 614-619
3. Brass, E. P., and Stabler, S. P. (1988) Biochem. J. 255, 155-159
4. Beck, W., Flavin, M., and Ochoa, S. (1957) J. Biol. Chem. 229, 997-1010
5. Stabler, S. P., Marcell, P. D., Podell, E. R., Allen, R. H., and Lindenbaum, J. (1984) Blood 64, Suppl. 1, 42a (abstr)
6. Brass, E. P., Tahiliani, A. G., Allen, R. H., and Stabler, S. P. (1990) J. Nutr. 120, 290-297
7. Brass, E. P., Allen, R. H., Ruff, L. J., and Stabler, S. P. (1990) Biochem. J. 266, 809-815
8. Krahenbuhl, S., Ray, D. B., Stabler, S. P., Allen, R. H., and Brass, E. P. (1990) J. Clin. Invest. 86, 2064-2061
9. Hoppel, C. L., DiMarco, J., and Tandler, B. (1979) J. Biol. Chem. 254, 4164-4170
10. Chance, B. (1959) in Ciba Foundation Symposium on the Regulation of Cell Metabolism (Wolfstonehame, G. E., and O'Connor, C. M., eds) pp. 91-129, J. & A. Churchill Ltd., London
11. Estabrook, R. W. (1967) Methods Enzymol. 10, 41-47
12. Blair, P. V., Oda, T., Green, D. E., and Fernandez-Moran, H. (1987) Biochemistry 26, 756-763
13. Hoppel, C. L., and Cooper, C. (1989) Arch. Biochem. Biophys. 283, 173-183
14. Hatify, Y. (1978) Methods Enzymol. 53, 11-14
15. Sottocasa, G. L., Kuylenstierna, B. O., Lars, E., and Bergstrand, A. (1967) J. Cell Biol. 32, 415-438
16. Wharton, D. C., and Tzagoloff, A. (1967) Methods Enzymol. 10, 245-250
17. Hoppel, C. L., and Cooper, C. (1968) Biochem. J. 107, 367-375
18. Sere, P. (1969) Methods Enzymol. 15, 9-11
19. Hatify, Y., and Stiggall, D. L. (1978) Methods Enzymol. 53, 21-27
20. Takemori, S., and King, T. E. (1964) Science 144, 852-853
21. Ragan, C. I. (1987) Mitochondria, A Practical Approach (Darley-
Electron Transport Chain Defects in HCCL-treated Rats

Usmar, V. M., Rickwood, D., and Wilson, M. T., eds) pp. 96–101, IRL Press, Oxford
22. Williams, J. N., Jr. (1964) Arch. Biochem. Biophys. 107, 537–548
23. Gornall, A. G., Bardawill, G. J., and David, M. M. (1949) J. Biol. Chem. 177, 751–766
24. Binder, M., Kolhouse, J. F., Van Horne, K. C., and Allen, R. H. (1982) Anal. Biochem. 125, 253–258
25. Dolphin, D. (1971) Methods Enzymol. 18, 34–52
26. Armitage, P., and Berry, G. (1987) Statistical Methods in Medical Research, 2nd Ed., Blackwell Scientific Publications, Oxford
27. Hatefi, Y. (1985) Annu. Rev. Biochem. 54, 1015–1019
28. Morgan-Hughes, J. A., Cooper, J. M., Holt, I. J., Harding, A. E., Schapira, A. H., and Clark, J. B. (1990) Biochem. Soc. Trans. 18, 523–526
29. Cooper, J. M., Schapira, A. H. V., Toscano, A., Harding, A. E., Morgan-Hughes, J. A., and Clark, J. B. (1990) Biochem. Soc. Trans. 18, 517–519
30. Hayes, J. D., Lecky, B. R. F., Landon, D. N., Morgan-Hughes, J. A., and Clark, J. B. (1984) Brain 107, 1165–1177
31. Kennaway, N. G., Buist, N. R. M., Darley-Usmar, V. M., Papadimitriou, A., Dimauro, S., Kelley, R. I., Capaldi, R. A., Blank, N. K., and D’Agostino, A. (1984) Pediatr. Res. 18, 991–999
32. Haller, R. G., Lewis, S. F., Estabrook, R. W., Dimauro, S., Servidei, S., and Foster, D. W. (1989) J. Clin. Invest. 84, 155–161
33. Chance, B., and Williams, G. R. (1955) J. Biol. Chem. 217, 429–438
34. Giuditta, A., and Singer, T. P. (1959) J. Biol. Chem. 234, 662–665
35. Ziegler, D., and Doeg, K. A. (1962) Arch. Biochem. Biophys. 97, 41–50
36. Cabrini, L., Landi, L., Pasquali, P., and Lenaz, G. (1981) Arch. Biochem. Biophys. 208, 11–19
37. Satav, J. G., and Katayre, S. S. (1982) Mol. Cell. Endocrinol. 28, 173–189
38. Hoppel, C. L., Kerr, D. S., Dahms, B., and Roessmann, U. (1987) J. Clin. Invest. 80, 71–77