As continuously monitored by firefly luciferase bioluminescence, rat liver mitochondria incubated under State 4 conditions slowly synthesized ATP by phosphorylation of endogenous adenine nucleotide. This ATP production was sensitive to uncouplers and respiratory inhibitors and was partially sensitive to atractyloside. No lag as long as 1 min occurred between complete inhibition of respiration and complete inhibition of ATP production. No lag was observed for inhibition by uncoupler or atractyloside. The lag after respiratory inhibition was shortened by concentrations of uncoupler well below fully uncoupling levels. During the lag, ATP was formed when exogenous ADP was added. The magnitude of ATP formation was a decreasing function of the time interval between the addition of respiratory inhibitor and the addition of ADP. Formation of more than 2 μmol of ATP/g of protein was consistently observed at intervals of 10 s or less. The initial rate of formation of this ATP approached the rate of oxidative phosphorylation in the absence of inhibition.

Safranin was employed to monitor membrane potential (ΔΨ). Under conditions where 2.25 μmol of ATP/g of protein was formed after 10 s of KCN inhibition, there was an accompanying 35% rapid decrease in ΔΨ as compared to a control with no ATP synthesis. By extrapolation, 100% of ΔΨ (the major component of the proton electrochemical gradient, ΔμH+) was equivalent to 6.4 μmol of ATP/g of protein. This high ATP equivalence of the energized state agrees well with predictions of the chemiosmotic hypothesis but appears to be inconsistent with a chemical coupling hypothesis or with more recently advanced microscopic versions of chemiosmotic coupling.

Mitochondria are generally recognized to become “energized” under certain conditions as, for example, during State 4 respiration. However, relatively few attempts have been made to determine the energy equivalence of mitochondria in the energized state. Based upon the ability of rat liver mitochondria to retain calcium ion after respiratory inhibition, Azzi and Chance (2) estimated that the energized state is equivalent to 0.3 μmol of ATP/g of mitochondrial protein. In earlier studies, Eisenhardt and Rosenthal (3, 4) calculated an energy equivalence of 0.8 μmol of ATP/g of protein from observations of an initial rapid phase of ATP formation (ATP jump) when ADP was added to energized mitochondria. However, this ATP jump has been subject to varying interpretations (5, 6) and could not be reproduced when ATP was measured continuously by a luciferase bioluminescence method (7). In view of the paucity of information concerning the energy equivalence and lifetime of the energized state in mitochondria, and the potential importance of such information to the mechanism of energy transduction, we have undertaken a study of the energized state employing the sensitive bioluminescence technique developed in our laboratory for the continuous measurement of ATP in suspensions of mitochondrial (7–9). We report here direct evidence for the lifetime and ATP equivalence of the energized state based upon ATP production by energized mitochondria after respiratory inhibition.

**MATERIALS AND METHODS**

Mitochondria from the livers of 200 to 250-g male Sprague-Dawley rats were isolated at 0–4°C by modification of Schneider’s procedure (10). Livers were quickly excised, cut into small pieces, and homogenized as a 30% (w/v) suspension in 0.25 M sucrose. The resulting homogenate was diluted to 10% (w/v) with sucrose and centrifuged for 10 min at 600 × g. The supernatant was carefully removed and centrifuged at 9750 × g for 15 min. The pellet was resuspended and sedimented three more times with centrifugations of 9750 × g for 10 min each. The final pellet was resuspended in sucrose to a concentration of 50 or 100 mg of protein/ml. Mitochondria routinely gave respiratory control ratios of 6 to 8 after addition of ADP (11).

Oxygen was measured polarographically (12), and ATP was measured continuously with firefly luciferase luminescence (7, 8). Luminescence was detected either by a Brice-Phoenix model 2000-D light-scattering photometer or by the photometer of an Amino DW-2a spectrophotometer. Vigorous magnetic stirring was provided throughout. A detailed account of the methods employed to quantitate the continuous luminescence signal is provided elsewhere (13).

The oxidation-reduction levels of cytochromes a+a3, c+c3, and b were measured by absorbance changes at the wavelength pairs 605-625 nm, 551-540 nm, and 562-575 nm or 430-410 nm, respectively, in an Amino DW-2a dual wavelength spectrophotometer. Membrane potential (ΔΨ) was monitored at the wavelength pair, 513-533 nm, in the presence of 10 μM safranin O as described by Åkerman and Wikstrom (15).

The basic reaction medium in all experiments contained 155 mM sucrose, 5 mM MgCl2, 10 units/ml of luciferase, 7.1 μM luciferin, 1 mg/ml of mitochondrial protein, and 11 mM KPO4 buffer, pH 7.4, 23°C. Respiratory substrate was either 5 mM 3-hydroxybutyrate or 5 mM sodium succinate plus 5 mM rotenone. Other additions to the reaction medium are indicated in the figure legends.

Protein was determined by a biuret procedure (16) using bovine serum albumin as standard. Purified firefly luciferase and synthetic firefly luciferin were obtained from Du Pont Corp. Safranin O was obtained from Fisher Scientific Co. FCCP was kindly donated by Dr. P. G. Heytler, DuPont Corp. Other reagents were obtained from the standard sources.

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*Nomencature of Chance and Williams (1).

The abbreviation used is: FCCP, carbonyl cyanide p-trifluoro-methoxyphenylhydrazone.
RESULTS

Endogenous ATP Production during State 4 Respiration—Rat liver mitochondria slowly released endogenously synthesized ATP during respiratory State 4 incubation. This release was monitored with firefly luciferase, an enzymatic probe of extramitochondrial ATP (Fig. 1). Because of product inhibition of the luciferase reaction, luminescence was not constantly proportional to ATP concentration, and it was necessary to use ATP standards to quantitate the luminescence signal at various time intervals (8, 9). During the first 2 to 3 min, however, the change in proportionality was relatively small, and luminescence was a reasonably accurate measure of ATP concentration without the use of exogenous ATP standards.

It is well known that isolated mitochondria rapidly and quantitatively convert exogenously added AMP or ADP to ATP under suitable incubation conditions. This observation extended to concentrations of adenine nucleotide as small as 1 μM or less in which case the ATP so formed appeared in addition to that generated from endogenous sources (data not shown). The phosphorylation of exogenous AMP was rapid. By contrast, in the absence of added nucleotide the appearance of extramitochondrial ATP was slow (Fig. 1). The latter finding suggested that endogenous ATP production was the result of phosphorylation of adenine nucleotide originally contained in an internal compartment.

ATP production during State 4 incubation was inhibited by atractyloside, an inhibitor of the ADP/ATP carrier (Fig. 2A). The onset of inhibition was virtually immediate, but inhibition was no greater than about 50% even at very high atractyloside concentrations (Fig. 2B). A similar onset of inhibition was obtained for atractyloside inhibition of active (State 3) phosphorylation, except that maximal inhibition was virtually 100% (11).

These observations are consistent with the mechanism proposed by Duee and Vignais (17) for oxidative phosphorylation of endogenous adenine nucleotide by rat liver mitochondria in a magnesium-containing medium very similar to ours. In freshly isolated mitochondria, adenine nucleotide is localized almost entirely in the matrix space. AMP is in excess of ADP, and there is very little ATP. At the beginning of State 4 incubation, ADP is rapidly phosphorylated to ATP while AMP is only slowly converted to ATP. The limiting step is the formation of ADP from AMP, since AMP itself is not a substrate for the ATP synthase of the inner membrane (7, 17, 18). In order for AMP to be converted to ADP, it must first leak out of the matrix space, whereupon it is transphosphorylated by adenylate kinase to ADP. The ADP so formed exchanges with intramitochondrial ATP via the ADP/ATP carrier and is then converted to ATP by oxidative phosphorylation. The ADP/ATP carrier-mediated step explains the partial atractyloside sensitivity of endogenous ATP formation during State 4. Thus, the overall rate of oxidative phosphorylation of endogenous adenine nucleotide and the rate of appearance of extramitochondrial ATP depend upon the rate at which AMP leaks out of the matrix space. In this respect the formation of endogenous ATP resembles ATP formation resulting from the slow, continuous addition of exogenous AMP or ADP.

Lag in Inhibition of ATP Production after Inhibition of Respiration—Endogenous State 4 ATP production was inhibited by cyanide and antimycin as well as by rotenone when using 3-hydroxybutyrate as respiratory substrate (Figs. 3 to 5). Action by these inhibitors on ATP synthesis, however, had an onset delay of up to 30 s or more. By contrast, the same concentrations of respiratory inhibitors produced nearly immediate inhibition of active (State 3) oxidative phosphorylation (Figs. 3 to 5). With endogenous State 4 ATP production the duration of the onset delay tended to decrease as respiratory inhibitor concentration increased, especially for antimycin, and to a lesser extent, for rotenone. These inhibitor concentrations (10 to 20 times greater than necessary for full inhibition) produced an activation of ATPase as was evidenced by an accelerated decline of luminescence.

Uncouplers, such as FCCP, also inhibited endogenous State 4 ATP production (Fig. 6). Unlike respiratory inhibitors, the onset of action by FCCP was immediate. At higher concentrations, FCCP caused a substantial, direct inhibition of the luminescence reaction itself as demonstrated by the rapid, concentration-dependent drop of luminescence immediately following FCCP addition. This direct inhibitory action could be demonstrated in the absence of mitochondria. Other uncouplers, such as dinitrophenol, dicoumarol, and carbonyl cyanide m-chlorophenylhydrazone, were also inhibitory to the luminescence reaction. Oxidative phosphorylation, however, was sensitive to lower concentrations of uncouplers than was luminescence.

The onset kinetics observed for the various respiratory inhibitors suggested the existence of an energized state that persisted for many seconds after the cessation of respiration.
Energized State of Mitochondria

Fig. 3. Luminescence recordings of potassium cyanide (KCN) inhibition of oxidative phosphorylation. A, KCN is added during State 4, and B, during State 3. Adenine nucleotide (AdN) (15 μM ADP and 121 μM AMP) is added in B after 2 min. Succinate is substrate.

Fig. 4. Luminescence recordings of antimycin inhibition of oxidative phosphorylation. A, antimycin is added during State 4, and B, during State 3. Reaction conditions are identical with Fig. 3. AdN, adenine nucleotide.

It was important to document, therefore, that respiratory inhibition in fact took place rapidly and completely under State 4 conditions. Since oxygen uptake was inhibited within the response time of the oxygen electrode (90% response in 10 s), the oxidation-reduction states of cytochromes \( b, c+c_1, \) and \( a+a_3 \) were examined utilizing dual beam spectrophotometry (Fig. 7). After addition of 1 mM KCN to mitochondria during State 4 incubation, all cytochromes immediately began to shift to a more reduced state. The half-times to maximal reduction were 8, 3, and 5 s, respectively, for cytochromes \( b, c+c_1, \) and \( a+a_3 \) (Table I). These experiments showed that the onset of respiratory inhibition by 1 mM KCN was quite rapid and occurred within the mixing time (~1 s) of the system. Since full reduction of the cytochromes proceeded rapidly as well, intrachain electron transfer is unlikely to explain the observed lag in the luminescence signal. Similar results were obtained for inhibition by 0.1 μg/ml of antimycin (Table I).

Uncouplers in increasing amounts shortened and then completely abolished the lag in luminescence after respiratory inhibition (Fig. 8A). The lag was considerably more sensitive to uncoupler than was respiratory rate. For example, 20 nM FCCP shortened the lag (as measured by the time interval between KCN addition and the point of greatest rate of decrease of luminescence) by 75%. However, this same concentration of FCCP stimulated respiration to only 25% of the maximal uncoupler-stimulated rate (Fig. 8B).

ATP Equivalence of the Energized State—If an energization hypothesis is correct, then discharge of the energized state should be accelerated by ADP coincident with the formation of ATP. This was our observation (Fig. 9). Adenine nucleotide as a mixture of ADP and AMP was added to the State 4 mitochondrial suspension at varying times following KCN addition. The result was ATP formation which decreased as the time between additions increased. The initial rate of this ATP formation approached the rate of ATP formation in the absence of inhibitor. After 10 s of KCN inhibition, the initial rate as determined by the slope of the
Fig. 7. Cytochrome reduction after respiratory inhibition with KCN. Recordings of absorbance at the wavelength pairs 605-625 nm (a+c), 551-540 nm (c+c1), and 562-575 nm (b) are shown from parallel experiments. Luminescence is shown in the bottom trace. Additions are: KCN, 1 mM; ATP, 1.0 μM. Succinate is substrate.

Table I
Oxidation-reduction changes of cytochromes following respiratory inhibition

| Inhibitor | Cytochrome |
|-----------|------------|
|           | a+c | c+c1 | b     |       |
| 1 mM KCN  | 0.00038 (5 s) | 0.00035 (8 s) | 0.0009 (8 s) | -0.0046 (8 s) |
| 0.1 μg/ml of Antimycin | 0 | -0.0015 (5 s) |       |       |

*Listed is the absorbance change following inhibitor addition at the stated wavelength pair. The half-time (t1/2) of this change is given in parentheses.

The question arises, how do these various manipulations affect ΔΨ? Under the conditions employed and using 10 mM phosphate, ΔΨ is composed primarily of ΔΨ (19). Therefore, we employed the electrogenic dye, safranin, to monitor ΔΨ (Fig. 12). Upon addition of mitochondria to a State 4 medium, ΔΨ rapidly reached a new and more negative steady state value that remained constant until oxygen was exhausted. This more negative steady state was rapidly and completely reversed by uncoupler (data not shown). KCN inhibition caused a much slower decrease of ΔΨ, since energy from ATP hydrolysis was used to sustain the electrochemical gradient (Fig. 12). When adenine nucleotide was added 10 s following KCN addition, there was a more rapid decrease of ΔΨ, especially during the initial few seconds. Comparison of these last two experiments shows that adenine nucleotide addition caused ΔΨ to drop more rapidly by an extra increment that corresponded to 35% of the full ΔΨ. Since 2.25 μmol of ATP/g of protein were produced as a result of the adenine nucleotide addition (Fig. 12b), and since this synthesis was associated with a decrease of 35% in ΔΨ, we calculate that 100% of ΔΨ is equivalent to 6.4 μmol of ATP/g of protein.
Energized State of Mitochondria

FIG. 9. ATP synthesis following KCN inhibition. Luminescence recordings are shown in A. KCN (2 mM) and adenine nucleotide (AdN) (15 μM ADP and 58 μM AMP) are added where indicated. In B, ATP formed is plotted versus the time interval between KCN and AdN addition. ATP formation is corrected for ATP contamination (0.3 μM) of AdN.

TABLE I
ATP synthesis after respiratory inhibition

| Inhibitor (concentration) | ATP synthesis (pmol/mg protein) | Initial rate of ATP production (μmol/min/mg protein) |
|--------------------------|---------------------------------|----------------------------------------------------|
| None                     | 119,82'                         |                                                   |
| KCN (1 mM)               | 2.11                            | 78                                                 |
| Antimycin (0.1 μg/ml)    | 1.78                            | 69                                                 |
| KCN (1 mM) + antimycin   | 1.81                            | 79                                                 |
| (0.1 μg/ml)              | 0.90                            | 41                                                 |

* Corrected for ATP contamination (0.2 μM) of adenine nucleotide.
* Calculated from the initial rate of luminescence increase after adenine nucleotide addition.
* 3-Hydroxybutyrate is substrate.

FIG. 10. ATP synthesis following respiratory inhibition by various agents. Additions where indicated during the luminescence recordings are: KCN, 1 mM; antimycin, 0.1 μg/ml; rotenone, 0.1 μM; adenine nucleotide (AdN), 20 μM ADP and 110 μM AMP. Succinate is substrate in the upper three traces. In the bottom trace, 3-hydroxybutyrate is substrate.

FIG. 11. ATP synthesis following inhibition with uncoupler or atractyloside (Atr). Additions where indicated are: FCCP, 0.4 μM; atractyloside, 2 μM; adenine nucleotide (AdN), 15 μM ADP and 58 μM AMP. The added ADP is contaminated by a small amount (0.3 μM) of ATP. Succinate is substrate.

DISCUSSION

The action of respiratory inhibitors on endogenous ATP production during State 4 permitted dissociation of oxidative activities from phosphorylative ones. A lag as long as 1 min occurred between complete inhibition of State 4 respiration...
Energized State of Mitochondria

The findings presented here provide direct experimental support for the concept of an energized state in mitochondria. Following inhibition of respiration the energized state discharges over a period of time represented by the lag interval. As expected, uncouplers “short circuit” the energized state and hasten its decomposition. The energized state may also be discharged in a manner coupled to ATP formation. We consistently observed that more than 2 μmol of ATP/g of protein could be formed that was coupled solely to the discharge of the energized state. Respiratory chain transformations following blockade of flux through the chain did not contribute to such ATP formation, since simultaneous inhibition at more than one site in the respiratory chain did not significantly lessen ATP formation, and since spectral measurements of the cytochromes demonstrated rapid responses to the inhibitors employed.

The magnitude of ATP formation coupled to the discharge of the energized state is probably an underestimation of the true energy equivalence of the energized state. As pointed out by Azzzone and coworkers (24), according to chemiosmotic theory there is a threshold value for \( \Delta G^\circ_m \), below which ATP formation cannot occur for given concentrations of ATP, ADP, and P. In agreement with this view, we observed that ATP synthesis could only be associated with 35% of the decrease in \( \Delta \Psi \) after respiratory inhibition. During State 4 incubation the phosphate potential, \( \Delta G_p \), reaches a value of 15 to 16 kcal/mol (25). After respiratory inhibition and adenine nucleotide addition, \( \Delta G_p \) dropped to about 9 to 9.5 kcal/mol in our experiments. This decrease of ~40% matches reasonably closely the observed 35% drop in \( \Delta \Psi \). On this basis we calculate that a 100% drop in \( \Delta \Psi \) is equivalent to 6.4 μmol of ATP/g of protein, this latter value representing the total energy equivalence of the energized state in rat liver mitochondria.

Although high values have been obtained from postillumination ATP synthesis by light-energized chloroplasts and bacterial chromophores (28, 29), our value for the energy equivalence of the energized state is an order of magnitude greater than previous reports for mitochondria. Azzzi and Chance (2), employing aequorin, a bioluminescent jellyfish protein, measured the movements of endogenous mitochondrial Ca\(^{2+}\) as an indicator of mitochondrial energization. They concluded that the lifetime and energy equivalence of the energized state in rat liver mitochondria were, respectively, 7 ± 2 s and 0.3 μmol of ATP/g of mitochondrial protein. In their experiments both high energy phosphate bonds of ATP need to be counted so that the energy equivalence is 0.6 μmol of ATP/g of protein if only ATP synthesis from ADP is considered. The discrepancy between our results and those of Azzzi and Chance is not readily explained. Considering our observation that the energized state is extremely sensitive to uncoupler, the shortened lifetime and energy equivalence observed by Azzzi and Chance could be due to a small amount of endogenous or introduced uncoupler. A more likely explanation, however, is that Ca\(^{2+}\) retention, like ATP synthesis, requires a threshold energy level and that Azzzi and Chance’s experiments measured the degree to which this threshold level was exceeded.

Eisenhardt and Rosenthal (3, 4) reported a similar value to Azzzi and Chance based upon the magnitude of an initial burst of ATP synthesis (ATP jump) when ADP was added to energized mitochondria. Since their ATP jump was insensitive to uncoupler, and since we were unable to reproduce their findings using the luminescence technique (7), it appears likely that their ATP jump phenomenon is due to factors other than mitochondrial energization as has been suggested (5, 6).

The value we calculate for the energy equivalence of the energized state represents the number of high energy bonds that would be formed throughout the decay of the energized state if there were no threshold free energy level for ATP synthesis, i.e. if ATP concentration were vanishingly small. Thus, our value for the energy equivalence of the energized state is not expressed in energetic units such as kilocalories or joules per g of protein. Rather, our calculation is representative of the accumulation of “high energy intermediates” during energization. If we assume, in accordance with classical chemical coupling theory, that the stoichiometry of high energy intermediate and ATP is one to one, then 6.4 μmol/g of...
protein of high energy intermediate accumulates during energization. This is nearly 2 orders of magnitude greater than the concentration of the respiratory chain enzymes, since in our preparations the concentration of the cytochrome aa₃ and cytochrome bc₁ complexes were, respectively, 0.12 and 0.08 μmol/g of protein (28). It is unlikely, therefore, that any direct chemical or conformational coupling mechanism could account for such a large amount of high energy intermediate.

For similar reasons, our data also appear to be inconsistent with various "microscopic" versions of chemiosmotic coupling (24, 29–31). In these versions, ATP synthesis is coupled within some microscopic domain to a proton gradient which is not in equilibrium with the bulk, transmembrane proton gradient. Thus, although a transmembrane proton gradient may be generated secondarily, the question is whether such a gradient can drive ATP synthesis at a rate sufficiently great to account for oxidative phosphorylation. Thayer and Hinkle (32) have shown that ATP synthesis driven by an artificial proton gradient in submitochondrial vesicles equals or exceeds the rate of oxidative phosphorylation by submitochondrial vesicles composed entirely of ApH or entirely of A*. These values are similar to direct measurements of energized mitochondria depending upon whether A⁺H⁺ is all A* and a total A⁺H⁺ of 270 mV, then the discharge of A⁺H⁺ to zero is the equivalent of 6.4 μmol of ATP/g of protein if A⁺H⁺ is all A* and 45 μmol of ATP/g of protein if A⁺H⁺ is entirely ΔΨ. Our observed energy equivalence of 6.4 μmol of ATP/g of protein is, therefore, consistent with A⁺H⁺- being comprised of about 15% ΔΨ and 85% ΔΨ. These values are similar to direct measurements of ΔΨ and ΔΨ for energized rat liver mitochondria under conditions of similar ionic strength and phosphate concentration (19). If the proton stoichiometry per coupling site is greater than two, as has recently been suggested (36–38, cf. also 19, 24, 34), then the relative contribution of ΔΨ to A⁺H⁺ would be greater, since more protons would be required for the synthesis of the same amount of ATP.

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REFERENCES
1. Chance, B., and Williams, G. R. (1956) Adv. Enzymol. 17, 65–134
2. Azzi, A., and Chance, B. (1969) Biochim. Biophys. Acta 189, 141–151
3. Eisenhardt, R. H., and Rosenthal, O. (1964) Science 143, 476–477
4. Eisenhardt, R. H., and Rosenthal, O. (1968) Biochemistry 7, 1327–1333
5. Van Dam, K. (1966) Biochim. Biophys. Acta 128, 337–343
6. Cross, R. L., and Boyer, P. D. (1975) Biochemistry 14, 392–396
7. Lemasters, J. J., and Hackenbrock, C. R. (1976) Eur. J. Biochem. 67, 1–10
8. Lemasters, J. J., and Hackenbrock, C. R. (1977) Biochemistry 16, 445–447
9. Schneider, W. C. (1948) J. Biol. Chem. 176, 259–266
10. Lemasters, J. J., and Sowers, A. E. (1979) J. Biol. Chem. 254, 1248–1251
11. Estabrook, R. W. (1967) Methods Enzymol. 10, 41–48
12. Lemasters, J. J., and Hackenbrock, C. R. (1978) Methods Enzymol. 57, 38–50
13. Chance, B. (1965) J. Biol. Chem. 240, 2729–2748
14. Åkerman, K. O. E., and Wikstrom, M. K. F. (1976) FEBS Lett. 68, 191–197
15. Gornall, A. G., Bardawill, C. J., and David, M. M. (1949) J. Biol. Chem. 177, 751–766
16. Deüe, E. D., and Vignais, P. V. (1969) J. Biol. Chem. 244, 3922–3940
17. Heldt, H. W., and Klingenberg, M. (1968) Eur. J. Biochem. 4, 1–8
18. Nicholls, D. G. (1974) Eur. J. Biochem. 50, 305–315
19. Meissner, H., and Klingenberg, M. (1968) J. Biol. Chem. 243, 3631–3639
20. Smith, C. M., Bryla, J., and Williamson, J. R. (1974) J. Biol. Chem. 249, 1497–1505
21. Akerboom, T. P. M., Krietisch, W. K. G., Kuntz, G., and Sies, H. (1979) FEBS Lett. 105, 90–94
22. Nijs, P. (1967) Biochim. Biophys. Acta 143, 454–461
23. Azzone, G. F., Massari, P., and Pozzan, T. (1977) Mol. Cell. Biochem. 17, 101–112
24. Slater, E. C., Rosing, J., and Mol, A. (1973) Biochim. Biophys. Acta 292, 534–553
25. Jagendorf, A. T. (1975) in Bioenergetics of Photosynthesis (Govindjee, ed) pp. 414–492, Academic Press, New York
26. Leiser, M., and Gromet-Elhanan, Z. (1975) J. Biol. Chem. 250, 84–89
27. Williams, R. J. P. (1961) J. Theor. Biol. 1, 1–13
28. Williams, R. J. P. (1978) Biochim. Biophys. Acta 505, 1–44
29. Rottenberg, H. (1978) FEBS Lett. 94, 295–297
30. Thayer, W. S., and Hinkle, P. C. (1975) J. Biol. Chem. 250, 5336–5345
31. Harris, D. A., Tscherner, V. V., and Radda, G. K. (1979) Biochim. Biophys. Acta 548, 72–84
32. Lemasters, J. J. (1980) FEBS Lett. 110, 96–100
33. Mitchell, P. (1966) Biol. Rev. (Cambridge) 41, 445–502
34. Brand, M. D., Reynafarje, B., and Lehninger, A. L. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 457–441
35. Reynafarje, B., Brand, M. D., and Lehninger, A. L. (1976) J. Biol. Chem. 251, 7442–7451
36. Alexandre, A., Reynafarje, B., and Lehninger, A. L. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 5296–5300