Knowles, A. F., and Guillory, R. J. (1970), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 29*, 895 Abs.
Knowles, A. F., Guillory, R. J., and Racker, E. (1971), *J. Biol. Chem. 246*, 2672.
Lardy, H. A., Johnson, D., and McMurray, W. C. (1958), *Arch. Biochem. Biophys. 78*, 587.
Lardy, H. A., Witonsky, P., and Johnson, D. (1965), *Biochemistry 4*, 552.
Lee, C.-P., and Ernster, L. (1965), *Biochem. Biophys. Res. Commun. 18*, 523.
Lowry, O. H., Rosebrough, N., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.
MacLennan, D. H., and Tzagoloff, A. (1968), *Biochemistry 7*, 1603.
Maurer, H. R. (1968), Disk Elektrophorese, Berlin, Walter de Gruyter & Co.
Penefsky, H. S. (1967), *J. Biol. Chem. 242*, 5789.
Pullman, M. E., Penefsky, H. S., Datta, A., and Racker, E. (1960), *J. Biol. Chem. 235*, 3322.
Racker, E. (1962), *Proc. Nat. Acad. Sci. U. S. 48*, 1659.
Racker, E. (1963), *Biochem. Biophys. Res. Commun. 10*, 435.
Racker, E., and Horstman, L. L. (1967), *J. Biol. Chem. 242*, 2547.
Racker, E., Pullman, M. E., Penefsky, H. S., and Silverman, M. (1961), *Proc. 5th Int. Congr. Biochem., Moscow 5*, 303.
Roberton, A. M., Holloway, C. T., Knight, I. G., and Beechey, R. B. (1966), *Biochem. J. 100*, 78P.
Roberton, A. M., Holloway, C. T., Knight, I. G., and Beechey, R. B. (1968), *Biochem. J. 108*, 445.
Schatz, G., Penefsky, H. S., and Racker, E. (1967), *J. Biol. Chem. 242*, 2552.
Senior, A. E., and Brooks, J. C. (1970), *Arch. Biochem. Biophys. 140*, 257.
Takayama, K., MacLennan, D. H., Tzagoloff, A., and Stoner, C. D. (1964), *Arch. Biochem. Biophys. 114*, 223.
Thompson, R. Q., Hoehn, M. M., and Higgins, C. E. (1961), *Antimicrob. Ag. Chemother.*, 474.
Tzagoloff, A. (1969a), *J. Biol. Chem. 244*, 5020.
Tzagoloff, A. (1969b), *J. Biol. Chem. 244*, 5027.
Tzagoloff, A. (1970), *J. Biol. Chem. 245*, 1545.
Tzagoloff, A., Byington, K. H., and MacLennan, D. H. (1968a), *J. Biol. Chem. 243*, 2405.
Tzagoloff, A., MacLennan, D. H., and Byington, K. H. (1968b), *Biochemistry 7*, 1596.
Walther, H. (1963), *Chem. Abstr. 58*, 2382*f*.
Weber, K., and Osborn, M. (1969), *J. Biol. Chem. 244*, 4406.

Effect of Antimycin A and 2-Heptyl-4-hydroxyquinoline *N*-Oxide on the Respiratory Chain of Submitochondrial Particles of Beef Heart†

J. R. Brandon,‡ J. R. Brocklehurst, and C. P. Lee*

ABSTRACT: Comparative studies of the effects of antimycin A and 2-heptyl-4-hydroxyquinoline *N*-oxide (HOQNO) on some of the chemical and physical properties of the respiratory chain of submitochondrial particles from beef heart have been made. (1) Antimycin A and HOQNO exhibit similar inhibition of both forward (NADH and succinate oxidases) and reversed (from ascorbate + PMS → NAD^+ supported by added ATP) electron flow of the respiratory chain. On a molar basis the amount of antimycin A required to give complete inhibition is approximately equal to the amount of cytochrome b_{561}. Antimycin A is 10 times more efficient than HOQNO. (2) Both compounds induce an absorption increase with a peak at 565.5 nm accompanied by a shoulder at 557–558 nm with either NADH- or succinate-reduced, terminally inhibited particles. The spectral changes titrate with antimycin A or HOQNO in the same way as the inhibitory effects, but somewhat less of either compound is required (on a mole to mole basis) to attain the maximal effect. (3) The spectral peak at 566 nm induced by antimycin A consists of two components: reduction of cytochrome b_{566} and a red shift of the reduced form of cytochrome b_{561}. HOQNO induces *only* the reduction of cytochrome b_{566}. (4) The antimycin-A-induced red shift is seen most clearly in a dithionite-reduced preparation where there is no interference from cytochrome b_{566}. (5) ATP induces a partial reduction of cytochrome b_{566} in the succinate–KCN-pretreated preparation. No red shift was observed.

It is now generally accepted that there are two major *b* cytochromes associated with the mitochondrial inner membrane which can be distinguished spectrophotometrically (Chance, 1952, 1958; Chance and Schoener, 1966; Chance *et al.*, 1966; Slater *et al.*, 1970a,b; Sato *et al.*, 1971a,b; Wikström, 1972) and potentiometrically (Dutton *et al.*, 1970, 1971; Wilson and Dutton, 1970; Rieske, 1971). One, cytochrome b_{561}, is characterized by a single α band at 561 nm at room temperature and a midpoint potential of +30 mV, and is fully reducible by succinate or NADH in anaerobiosis.

† From the Johnson Research Foundation, University of Pennsylvania, Philadelphia, Pennsylvania 19104. *Received September 29, 1971.* This investigation has been supported by grants from the U. S. National Science Foundation (NSF GB23253), and the U. S. National Institutes of Health (NIH, GM12202). J. R. Brandon is a recipient of an NIH postdoctoral fellowship (5F-02GM49877). J. R. Brocklehurst is a recipient of a Wellcome Research travel grant. C. P. Lee is an NIH career development awardee, (1-K4-GM38822).

‡ Present address: Department of Chemistry, Ohio State University, Columbus, Ohio 43210.

The other, cytochrome b_{566}, is characterized by a double α band with maxima at 566 and 558 nm at room temperature, and a midpoint potential of -30 mV, and cannot be reduced by succinate or NADH unless either antimycin A or dithionite is also present. These are, respectively, cytochromes *b* and b_i in the notation of Slater *et al.* (1970a,b) and cytochromes b_K and b_T in the notation of Chance *et al.* (1970).

Antimycin A has been shown to be a potent inhibitor of the mitochondrial electron transfer chain (Ahmad *et al.*, 1950; Potter and Reif, 1952) acting in the span between cytochromes *b* and c_1 (Chance, 1952; Chance and Williams, 1956). The amount of antimycin A required to inhibit completely succinate (or NADH) oxidase activity is stoichiometric with the cytochrome *b* content being 1:1 for substrate-reducible cytochrome *b* (Chance, 1958; Estabrook, 1962) and 1:2 for dithionite-reducible cytochrome *b* (Pumphrey, 1962; Rieske *et al.*, 1967; Takemori and King, 1964; Berden and Slater, 1970). Antimycin A also causes marked changes in the cytochrome *b* spectra, particularly an increase in absorption at 565 nm (Chance, 1952; Chance and Williams, 1956; Chance, 1958; Slater and Colpa-Boonstra, 1961; Pumphrey, 1962). Similar spectral changes can also be induced by ATP. Slater *et al.* (1970b) further observed that the ATP- and antimycin-A-induced spectral effects are additive and inferred that they are due to a red shift of the absorption peaks of the two *b* cytochromes. The interpretation of the ATP- and antimycin-A-induced spectral changes has been a matter of controversy. Slater *et al.* (1970b) concluded that antimycin A affects cytochrome b_i, while ATP affects cytochrome *b*, and that the absorption increase at 565 nm is caused by the formation of high-energy forms of the two *b* cytochromes. This interpretation has been challenged by Sato *et al.* (1971a,b). The latter investigators concluded that ATP induces an energy-dependent reduction of cytochrome b_T (b_i) but has no effect on cytochrome b_K (*b*), while antimycin A induces the reduction of cytochrome b_T (b_i) by lowering the effective oxidation–reduction potentials expressed on the two *b* cytochromes by the substrate, and may also induce a small red shift of cytochrome b_K (*b*).

An inhibitor which appears to have its site of action in the same region as antimycin A is 2-heptyl-4-hydroxyquinoline *N*-oxide (HOQNO) (Lightbown and Jackson, 1956). Inhibition of succinate oxidase activity by HOQNO is reportedly less effective than that by antimycin A by factors ranging from 10-fold (Löw and Vallin, 1963) to 40-fold (Fynn, 1969). The effect of HOQNO on cytochrome *b* spectra has been shown to be qualitatively similar to that of antimycin A (Chance, 1958), but no systematic study has been made.

It was the purpose of the present study to make a systematic comparison of the effects of antimycin A and HOQNO on the cytochromes *b* spectra of phosphorylating submitochondrial particles from beef heart mitochondria in the energized and nonenergized states. In particular, attempts were made to distinguish whether the inhibitors affect one or both of the *b* cytochromes, and what relationship, if any, these effects have to those caused by ATP. A quantitative correlation between the spectral effects and the inhibition of both forward and reversed electron flow by antimycin A and HOQNO has also been made.

Methods and Materials

Phosphorylating electron transport particles derived from "heavy" beef heart mitochondria after sonication in the presence of ATP and Mg^{2+} (Löw and Vallin, 1963) were used throughout the present studies.

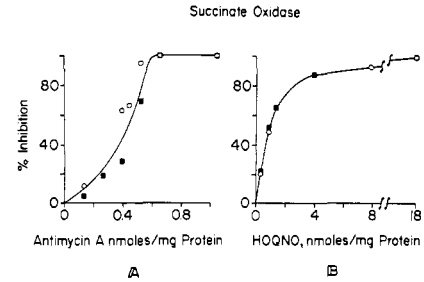

FIGURE 1: Inhibition of succinoxidase by antimycin A and HOQNO. The reaction mixture consisted of 170 mM sucrose, 30 mM Tris-acetate buffer, pH 7.5, 1.5 mg of protein of Mg^{2+}–ATP particles. The reaction was started by the addition of 3.3 mM Tris-succinate. Total volume: 3.0 ml; temperature, 25°. Where indicated, varying amounts of antimycin A (A) or HOQNO (B) were added after succinate until a steady and constant rate was reached. (○) and (■) are in the absence and presence of 1.7 μM FCCP, respectively. The rates of uninhibited succinate oxidase were 196 and 325 nmoles per min per mg of protein in the absence and presence of FCCP, respectively.

Succinate oxidase was measured polarographically with a Clark oxygen electrode. NADH oxidase and the energy-linked reduction of NAD^+ were measured spectrophotometrically with a Zeiss recording spectrophotometer by following the absorbance changes at 340 nm. Difference spectra of the pigments of the mitochondrial respiratory chain were determined by means of a split beam scanning and recording spectrophotometer (Chance, 1951; Yang and Legallais, 1954) with an expanded wavelength scale. When ATP was employed as the energy-yielding substrate for the spectral titrations, phosphoenolpyruvate and pyruvate kinase were used as an ATP-regenerating system to maintain a constant steady-state level of ATP. Cytochrome *b* content was estimated spectrophotometrically using an extinction coefficient of 20 mM^{-1} cm^{-1} at 561 minus 575 nm.

Protein content was determined by the biuret (Gornall *et al.*, 1949) method with crystalline bovine serum albumin as standard.

Antimycin A and HOQNO were obtained from Sigma, FCCP (carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone) was a gift of Dr. P. Heytler, E. I. duPont de Nemours and Co., Wilmington, Del. Other chemicals of the purest grades available were obtained commercially. Glass redistilled water was used throughout.

Results and Discussion

NADH and Succinate Oxidase Activities. The effect of antimycin A and HOQNO on the succinate oxidase and the NADH oxidase activities of submitochondrial particles from beef heart are shown in Figures 1 and 2, respectively. Approximately ten times more HOQNO on a molar basis is required than antimycin A to give virtually complete inhibition, in agreement with the work on antimycin A and 2-nonyl-HOQNO reported by Löw and Vallin (1963) for submitochondrial particles from beef heart, and by Takemori and King (1964) for the purified succinate cytochrome *c* reductase. In the case of antimycin A approximately 0.5–0.6 nmole/mg of particle protein are required to inhibit either activity completely. This value correlates well with the cytochrome *b* content (cytochrome b_{561}) in these particle preparations as estimated from the substrate-induced spectral changes at 561

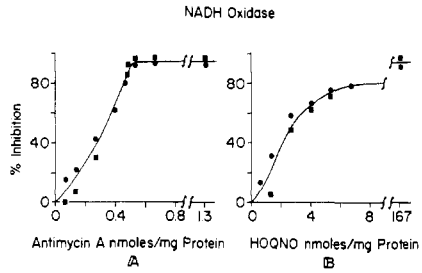

FIGURE 2: Inhibition of NADH oxidase by antimycin A and HOQNO. The reaction mixture consisted of 170 mM sucrose, 30 mM Tris-acetate buffer (pH 7.5), and 200 μM NADH. The reaction was started by the addition of 0.3 mg of protein of Mg^{2+}–ATP particles. Total volume: 3.0 ml, temperature 25°. Where indicated varying amounts of antimycin A (A) and HOQNO (B) were added until a steady and constant rate was reached. (●) and (■) are in the absence and presence of 1.7 μM FCCP, respectively. The rates of uninhibited NADH oxidase were 355 and 572 nmoles per min per mg of protein in the absence and presence of FCCP, respectively.

minus 575 nm under anaerobic conditions. No significant differences in the titration profiles were observed regardless of whether the particles were in an energy-rich or energy-poor state.

Reversed Electron Transfer. Similar effects of antimycin A and HOQNO are observed for the ATP-supported reversed electron flow from ascorbate + PMS to NAD^+ (Figure 3). It should be noted that at the concentrations of antimycin A and HOQNO employed, there is essentially no inhibition of the ATP-supported reversed electron transfer from succinate to NAD^+. This is further demonstrated (Figure 3) by the restoration of the rate of reduction of NAD^+ upon the addition of succinate (dotted line) to the system where the rate of reduction by ascorbate + PMS was completely inhibited by antimycin A or HOQNO. This observation is in full agreement with the idea that the site of action of antimycin A or HOQNO is on the pathway of reversed electron flow from ascorbate + PMS to NAD^+, but not that from succinate to NAD^+ (Löw and Vallin, 1963; and also Ernster and Lee, 1964).

We may therefore conclude that antimycin A and HOQNO act similarly on the electron-transport properties of the

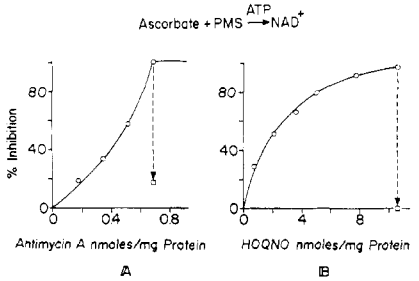

FIGURE 3: Effect of antimycin A and HOQNO on the ATP-supported reversed electron transport. The reaction mixture consisted of 170 mM sucrose, 30 mM Tris-acetate buffer (pH 7.5), 6.7 mM $MgSO_4$, 3.3 mM ATP, 8.3 mM Na ascorbate, 1.3 μM PMS, 3.3 mM KCN, and 1.5 mg of particle protein. The reaction was started by the addition of 1 mM NAD^+. Varying amounts of antimycin A (A) or HOQNO (B) were added subsequently until a steady and constant rate was reached. The dotted line indicates that the rate of NAD^+ reduction was recovered by 80% (A) or 100% (B) upon the addition of 3.3 mM Tris-succinate. The rate of uninhibited NAD^+ reduction by ascorbate + PMS or succinate was 81 nmoles/min per mg of protein at 24°.

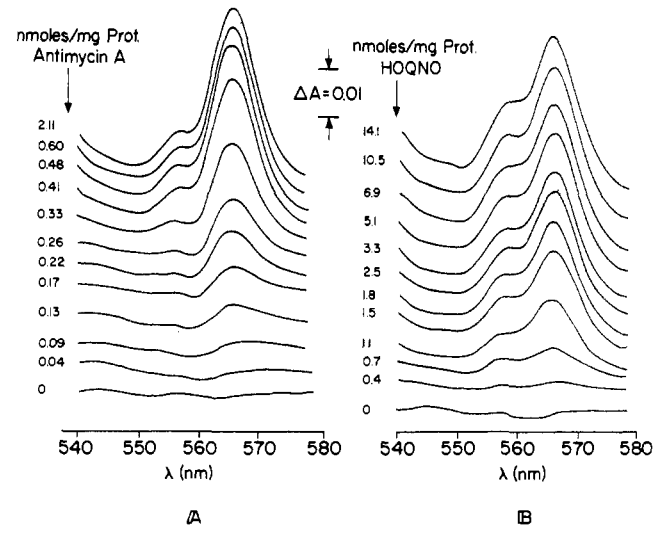

FIGURE 4: Difference spectra titrations with antimycin A (A) and HOQNO (B). The reaction mixture consisted of 170 mM sucrose, 30 mM Tris-acetate buffer (pH 7.5), 6.7 mM $MgSO_4$, 6.7 mM Tris-succinate, 6.7 mM KCN, 3.0 mM ATP, 100 μg of pyruvate kinase, 5 mM phosphoenolpyruvate and 30 mg of particle protein, total volume 6.0 ml; 3.0 ml of the suspension was then placed in the measuring cell, the remainder in the reference cell. A base line was recorded. Varying amounts of antimycin A (A) or HOQNO (B) were then added to the measuring cell and the difference spectra were recorded.

respiratory chain of particles as estimated from either forward or reversed electron flow, but that antimycin A is 10 times as efficient as HOQNO.

Spectral Changes. Substrate (NADH or succinate)-reduced terminally inhibited particles were titrated with antimycin A and HOQNO. Difference spectra (from 540 to 580 nm) were taken between samples in the presence and absence of varying amounts of antimycin A (Figure 4A) and HOQNO (Figure 4B). It is clearly seen that both antimycin A and HOQNO induce an increase in absorption with a peak at 565.5 nm and a shoulder at 557–558 nm. Figure 5 presents the plots of the absorbance changes at 565.5 nm as a function of antimycin A or HOQNO concentrations, where 575 nm was used as the reference wavelength. It is shown that as in the inhibition plots (Figures 1–3), HOQNO is approximately 10 times less efficient than antimycin A. In these spectral titrations, however, somewhat less (about 30%) of either compound is required to give maximal increase in absorption. The titer of antimycin A for maximal spectral increase is in agreement with that reported by Slater *et al.* (1970b). The extent of this spectral increase for particles in the presence of an energy supply (ATP) (open circles) is consistently smaller (5–10%)

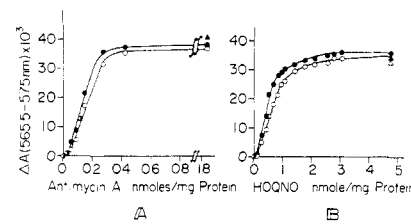

FIGURE 5: Titration curves for the spectral changes induced by antimycin A and HOQNO. Conditions were as in Figure 4. (○—○) and (●—●) were in the absence and presence of 5 μM FCCP, respectively. (△,▲) Dithionite was added at the end of each titration.

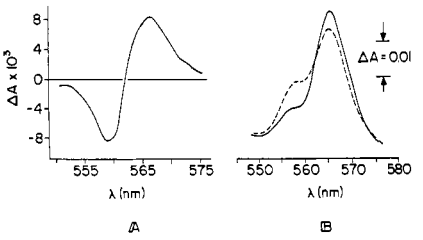

FIGURE 6: (A) Difference spectrum of particles fully inhibited by antimycin A and particles fully inhibited by HOQNO. Conditions were as in Figure 4 except that in the measuring cell 15 mg of protein of particles was treated with 30 nmoles of antimycin A, whereas in the reference cell the same amount of particles was treated with 210 nmoles of HOQNO. In B, the difference spectra between succinate + KCN pretreated particles in the presence and absence of 30 nmoles of antimycin A/15 mg of protein (solid line), and in the presence and absence of 210 nmoles of HOQNO/15 mg of protein (dotted line) are shown for comparison.

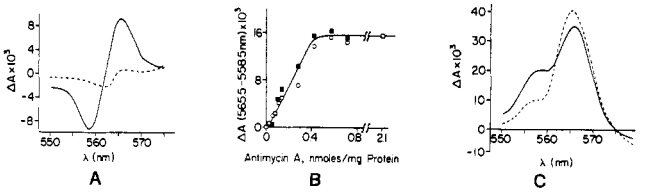

FIGURE 7: Difference spectra of particles fully reduced with sodium dithionite in the presence of antimycin A and of HOQNO. The reaction mixture consisted of 170 mM sucrose, 30 mM Tris-acetate buffer (pH 7.5), 6.7 mM $MgSO_4$, 3.3 mM KCN, 3.3 mM sodium succinate, 1.5 mM ATP, 100 μg of pyruvate kinase, 5 mM phosphoenolpyruvate, 30 mg of particle protein, and a sufficient amount of solid sodium dithionite until complete reduction was attained, total volume 6.0 ml; 3 ml of the suspension was placed in the measuring cell and the remainder in the reference cell. A base line was recorded; 30 nmoles of antimycin A (solid line) or 210 nmole of HOQNO (dotted line) were added to the measuring cell and the difference spectra (A) were recorded. The titration curve with varying amounts of antimycin A is shown in B. (■) and (○) were in the presence and absence of 5 μM FCCP, respectively. For comparison the difference spectra of dithionite + succinate minus succinate (solid line) and antimycin A + succinate minus succinate (dotted line) treated particles are shown in (C).

than that in the absence of energy. This difference can be accounted for by the fact that, in the presence of an energy supply, part of the total absorption increase at 565.5 nm had occurred *via* reversed electron transfer (*cf.* Ernster and Lee, 1964), prior to the addition of antimycin A or HOQNO (*cf.* also Figure 8). The total increase in absorption at 565.5 nm induced by HOQNO corresponds to the portion of the *b* cytochrome (approximately 40%) which is not reducible by substrate, but which can be reduced by dithionite (cytochrome b_{566}). The absorption increase induced by antimycin A is larger by approximately 15% (*cf.* Figure 7C).

It is interesting to note that the 557-nm shoulder (*cf.* Figure 4) is much more pronounced in the case of HOQNO-treated preparations than that of antimycin-A-treated preparations, and, in fact, at low concentrations of antimycin A there is an apparent dip at 558 nm. This suggests that a red shift may be occurring in the case of antimycin-A-treated preparations, but not in the case of HOQNO-treated preparations.

In order to differentiate the spectral changes induced by the two compounds, a difference spectrum between particles fully inhibited with antimycin A and particles fully inhibited with HOQNO was taken as shown in Figure 6A. It can be seen that there is an apparent shift from 558.5 to 565.5 nm, which amounts to approximately 10–15% of the absorption increase at 565 nm induced by dithionite treatment of the substrate-reduced particles. It should be added that virtually identical spectra were observed both in the energized and nonenergized particles. Figure 6B shows a superimposition of spectra for particles fully inhibited by antimycin A and by HOQNO as indicated.

To confirm this apparent shift, particles chemically reduced (dithionite) in the presence or absence of substrate were titrated with antimycin A and HOQNO. Virtually no effect was observed with HOQNO (Figure 7A, dotted line). The shift induced by antimycin A is shown in Figure 7A (solid line) along with the titration curve for the shift (Figure 7B). It can be seen that the shift parallels the absorption increase at 566 nm induced by antimycin A in the substrate-reduced particles. For comparison, the antimycin-A- and dithionite-induced spectral changes (550–580 nm) in the substrate-reduced particles are shown in Figure 7C. It should be noted that the same extent and amplitude of the shift are seen (*cf.* Figures 6A and 7A) regardless of the electron donor in contrast to the observations of Bryla *et al.* (1969) who reported a larger antimycin-A-induced shift when succinate was the reductant than when dithionite was used. No effect of antimycin A was observed with particle preparations in the absence of substrate or reducing agents. From these data we may therefore conclude that the apparent red shift results from an interaction between antimycin A and reduced *b* cytochrome(s) in agreement with the conclusions of Pumphrey (1962), Rieske (1971), and Sato *et al.* (1971b). Owing to the relatively wide bandwidths of the absorption changes, these data do not permit us to estimate the extent of the shift exactly. The spectra have been examined at liquid nitrogen temperature where the bandwidths are narrower. These results indicate a true red shift of only 2–3 nm, probably from 561 to 563 nm.

Effect of ATP on the b Cytochromes. ATP induces an absorption increase with a peak at 566 nm accompanied by a shoulder at 558 nm in the succinate + KCN pretreated particle preparation (Figure 8). Oligomycin or FCCP abolishes it completely (not shown). Similar results have also been observed with pigeon heart particles by Sato *et al.* recently (1971a,b). Sato *et al.* have concluded that the ATP-induced spectral change represents the reduction of cytochrome b_{566}. On the other hand, Slater *et al.* (1970b) have concluded that such a spectral change arises from the high-energy form of cytochrome b_{561}.

In order to clarify the ATP-induced spectral changes further we have used the absorbance ratio: $\Delta A_{566-575}/\Delta A_{558-575}$ as a tool for analysis of our data. The values obtained for various treatments of the succinate + KCN pretreated particles are

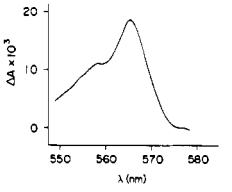

FIGURE 8: ATP-induced spectral change of Mg^{2+}–ATP particles. The reaction mixture consisted of 170 mM sucrose, 30 mM Tris-acetate buffer (pH 7.5), 6.7 mM $MgSO_4$, 3.3 mM sodium succinate, 3.3 mM KCN, 30 mg of particle protein, and 3 mM ATP, total volume 6.0 ml; 3.0 ml of the suspension was delivered into the measuring cell and the remainder into the reference cell. A base line was recorded. The difference spectrum was then recorded after the addition of 5 μM FCCP to the reference cell.

TABLE I: Effects of Various Reagents on the Spectral Changes of the Succinate + KCN Pretreated Particles.[a]

Treatment	Ratio ($\Delta A_{566-575}/\Delta A_{558-575}$)
Dithionite	1.7
Antimycin A	4.1
HOQNO	1.9
ATP	1.7

[a] Data are taken from the following figures: dithionite, Figure 7C; antimycin A, Figure 7C; HOQNO, Figure 6B; ATP, Figure 8.

shown in Table I. Taking the dithionite case as the base, a significant difference appears only in the antimycin-A-treated particles. This increase in the ratio arises from the contribution of a red shift of cytochrome b_{561} induced by antimycin A (*cf.* Figure 7A). The fact that virtually identical values were observed in the cases of ATP and HOQNO suggests that there is no ATP-induced shift and supports our contention that HOQNO does not induce a red shift (*cf.* Figure 7A).

The mechanism by which antimycin A and HOQNO induce an increase in reduction of cytochrome b_{566} is unclear. The obvious one would be an antimycin-A- (or HOQNO-) induced rise in the midpoint potential of cytochrome b_{566} rendering it reducible by substrate. However, there are conflicting reports of an antimycin-A-induced midpoint potential change. Urban and Klingenberg (1969) and Dutton *et al.* (1972) failed to observe a change using two different techniques. In contrast Rieske (1971) has obtained evidence for an antimycin-A-induced potential change. Rieske suggests that the redox state of a component X which is intimately associated with the b–c_1 region of the respiratory chain controls the midpoint potential of cytochrome b, and that antimycin A interacts with X. The stoichiometry indicates that there is only one antimycin A binding site per respiratory chain. Our data suggest that HOQNO binds at the same (or a very similar) site as antimycin A but with a lower affinity. If both inhibitors affect the redox state of cytochrome b_{566} by binding to X, then the spatial location of X must be such that the larger antimycin A molecule is close enough to cytochrome b_{561} to perturb its absorption spectrum. This possibility is now under investigation.

It should be noted that despite the requirement of energy for the reduction of cytochrome b_{566}, the data presented here do not allow us to evaluate the functional role of this cytochrome in the primary process of energy coupling at site II as proposed by Slater *et al.* (1970b), Wilson and Dutton (1970), and Chance *et al.* (1970).

Acknowledgments

The authors wish to thank Misses Basia Cierkosz and Sharon Askew for their valuable technical assistance.

References

Ahmad, K., Schneider, H. G., and Strong, F. M. (1950), *Arch. Biochem. 28*, 281.
Berden, J. A., and Slater, E. C. (1970), *Biochim. Biophys. Acta 216*, 237.
Bryla, J., Kaniuga, Z., and Slater, E. C. (1969), *Biochim. Biophys. Acta 189*, 317.
Chance, B. (1951), *Rev. Sci. Instr. 22*, 619.
Chance, B. (1952), *Nature (London) 169*, 215.
Chance, B. (1958), *J. Biol. Chem. 233*, 1223.
Chance, B., Lee, C. P., Schoener, B. (1966), *J. Biol. Chem. 241*, 4574.
Chance, B., and Schoener, B. (1966), *J. Biol. Chem. 241*, 4567.
Chance, B., and Williams, G. R. (1956), *Advan. Enzymol. 17*, 65.
Chance, B., Wilson, D. F., Dutton, P. L., and Erecinska, M. (1970), *Proc. Nat. Acad. Sci. U. S. 66*, 1175.
Dutton, P. L., Lindsay, J. G., and Wilson, D. F. (1972), *in* Biochemistry and Biophysics of Mitochondrial Membranes, Azzone, G. F., and Siliprandi, N., Ed., New York, N. Y., Academic Press (in press).
Dutton, P. L., Wilson, D. F., and Lee, C. P. (1970), *Biochemistry 9*, 5077.
Dutton, P. L., Wilson, D. F., and Lee, C. P. (1971), *Biochem. Biophys. Res. Commun. 43*, 1186.
Ernster, L., and Lee, C. P. (1964), *Annu. Rev. Biochem. 33*, 729.
Estabrook, R. W. (1962), *Biochim. Biophys. Acta 60*, 236.
Fynn, G. H. (1969), *Biochim. Biophys. Acta 180*, 244.
Gornall, A. G., Bardawill, C. J., and David, M. M. (1949), *J. Biol. Chem. 177*, 751.
Lightbown, J. W., and Jackson, F. L. (1956), *Biochem. J. 63*, 130.
Löw, H., and Vallin, I. (1963), *Biochim. Biophys. Acta 69*, 361.
Potter, V. R., and Reif, A. R. (1952), *J. Biol. Chem. 194*, 287.
Pumphrey, A. M. (1962), *J. Biol. Chem. 237*, 2384.
Rieske, J. S. (1971), *Arch. Biochem. Biophys. 145*, 179.
Rieske, J. S., Baum, H., Stoner, C. D., and Lipton, S. H. (1967), *J. Biol. Chem. 242*, 4854.
Sato, N., Wilson, D. F., and Chance, B. (1971a), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 15*, 209.
Sato, N., Wilson, D. F., and Chance, B. (1971b), *Biochim. Biophys. Acta 253*, 88.
Slater, E. C., and Colpa-Boonstra, J. P. (1961), *in* Haematin Enzymes, Part 2, Falk, J. E., Lemberg, R., and Morton, R. K., Ed., Oxford, Pergammon Press, p 575.
Slater, E. C., Lee, C. P., Berden, J. A., and Wegdam, H. J. (1970a), *Nature (London) 226*, 1284.
Slater, E. C., Lee, C. P., Berden, J. A., and Wegdam, H. J. (1970b), *Biochim. Biophys. Acta 223*, 354.
Takemori, S., and King, T. E. (1964), *J. Biol. Chem. 239*, 3546.
Urban, P. F., and Klingenberg, M. (1969), *Eur. J. Biochem. 9*, 519.
Wikström, M. K. F. (1972), *in* Biochemistry and Biophysics of Mitochondrial Membranes, Azzone, G. F., and Siliprandi, N., Ed., New York, N. Y., Academic Press (in press).
Wilson, D. F., and Dutton, P. L. (1970), *Biochem. Biophys. Res. Commun. 39*, 59.
Yang, C. C., and Legallais, V. (1954), *Rev. Sci. Instr. 25*, 801.