Oxidation–Reduction Potentials of Cytochromes in Mitochondria*

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ABSTRACT: Anaerobic techniques have been developed to permit the simultaneous potentiometric and spectrophotometric assay of oxidation–reduction of cytochromes in the mitochondrial membrane over a continuous potential range from +400 to −200 mV. The oxidation–reduction midpoint potentials (E_m) at pH 7.2 of the cytochromes of beef heart mitochondrial preparations are as follows: a_3, +365 mV; a, +205 mV; $c + c_1$, +227 mV; b, +38 mV. Two other membrane-bound components, which like cytochrome b undergo spectral changes at 430 and 562 nm during oxidation and reduction, have E_m values +125 and −103 mV at pH 7.2. In pigeon heart mitochondria cytochrome $c + c_1$ has E_m +233 mV at pH 7.2, and two b-type cytochromes have E_m values −15 and −100 mV at pH 8.1. The E_m of soluble cytochrome c is sensitive to the nature of the buffer in which it is dissolved; the E_m is lowered by as much as 60 mV on binding inside beef heart submitochondrial particles. Isolated cytochrome c_1, E_m +225 mV (pH 7.0), has a similar E_m to that observed in the mitochondrial membrane.

Considerable effort has been expended in determining the standard oxidation–reduction potentials of the enzymes of respiratory systems since, in general, this property provides some idea of the sequential arrangement of the electron carriers in the chain. But more important, it provides a physical-chemical basis for examination of the processes of energy conservation from the thermodynamic standpoint. Much of the work on the carriers of mammalian respiratory systems has been done on isolated, solubilized, or purified preparations of cytochrome oxidase (Ball, 1938; Wainio, 1955; Minnaert, 1965; Tzagoloff and Wharton, 1965), and cytochromes c (Ball, 1938; Rodkey and Ball, 1950; Henderson and Rawlinson, 1956) and c_1 (Green *et al.*, 1960). Besides variations in the reported midpoint potential values, particularly with mammalian cytochrome b (*cf.* Ball, 1938; Sekuzi and Okunuki, 1956; Feldman and Wainio, 1960; Rieske, 1969), there are several instances concerning b-type cytochromes in which significant differences in the midpoint potentials of the bound and free forms have been found (Goldberger *et al.*, 1962; Kawai *et al.*, 1963). There is therefore some room for doubt with respect to both the methods of determination and to the values obtained from isolated preparations. The more recent methods which have been employed to determine the midpoint potentials of cytochrome b bound in the mitochondrial membrane have generally been comparative (Holton and Colpa-Boonstra, 1960; Straub and Colpa-Boonstra, 1962; Urban and Klingenberg, 1969), using equilibrium *via* succinate dehydrogenase of succinate:fumarate ratios. This method provides only a limited potential range (about 60 mV) in which the measurements can be deemed reliable. They are also subject to some possible errors, at least in the intact mitochondria, arising from fumarase activity and also to membrane permeability.

Caswell (1968) and Caswell and Pressman (1969) used the device of simultaneous and continuous readout of absorbance and oxidation–reduction potential changes of the oxidation state of cytochromes in aerobic cyanide-inhibited mitochondria. They employed catalytic amounts of *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine which was exceptional in its rapid rate of mediation between the electrode and cytochrome c, and permitted experiments to be performed in the presence of oxygen; even so, cyanide was necessary to slow down the rate of respiration. Other known dyes in low concentrations are not as suitable for use in aerobic systems, since they do not mediate equilibration of the cytochrome with electrode rapidly enough, or react directly with molecular oxygen.

In this communication we have employed a method (Wilson and Dutton, 1970a,b; Dutton, 1970) which allows simultaneous potentiometric and spectrophotometric assay, but under strictly anaerobic conditions; this obviates the use of respiratory inhibitors and extends the approach to a choice of many dyes. This in turn extends the potential range in which reliable potentiometric assay can be made for the determination of the oxidation–reduction properties of the respiratory carriers in the mitochondrial membrane.

Materials and Methods

Beef heart mitochondria were prepared by the method of Löw and Vallin (1963). MgATP submitochondrial particles (MAsp)[1] and EDTA submitochondrial particles (Esp) were prepared from beef heart mitochondria according to the respective methods of Löw and Vallin (1963) and Lee and Ern-

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[1] Abbreviations used are: S-10, 5-chloro-3-(*p*-chlorophenyl), 2′,4′,5′-trichlorosalicylanilide. MAsp and Esp represent MgATP and EDTA beef heart submitochondrial particles, respectively; and BHM, PHM, and RLM represent beef heart, pigeon heart, and rat liver mitochondria, respectively. E_h is the electrical potential; E_{mx} the midpoint potential of an oxidation–reduction couple at pH x; n is the number of electrons transferred in an oxidation–reduction reaction (Clark, 1960).

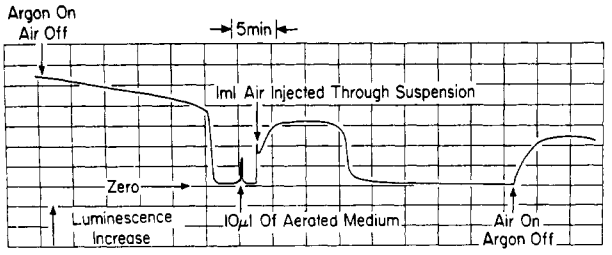

FIGURE 1: The luminescence of *A. fischeri* in the anaerobic cuvet. The rate of oxygen consumption by the *A. fischeri* was 7.2 μmole/min. Further details are in the text.

ster (1967). Rat liver mitochondria were prepared as previously described (Wilson, 1969) but washed twice with 0.12 M KCl to remove contaminating hemoglobin (Jacobs *et al.*, 1965). Pigeon heart mitochondria were prepared by the method of Chance and Hagihara (1961).

Horse heart cytochrome *c* (grade VI) was obtained from Sigma Chemical Co. Cytochrome c_1, isolated from beef heart, was a generous gift from Dr. T. E. King. The uncoupler, S-10, was kindly provided by Dr. Philip Hamm.

The oxidation–reduction mediators used to act between the membrane-contained cytochromes and platinum electrode were at pH 7.0 as follows: potassium ferricyanide, E_m +430 mV, n = 1 (Baker Chemical Co., Philadelphia, Pa.); *N,N,N',N'*-tetramethyl-*p*-phenylenediamine E_m +260 mV, n = 2 (Eastman Organic Chemicals, Rochester, N. Y.); diaminodurol, E_m +240 mV, n = 2 (generous gift from Dr. L. Ernster); phenazine methosulfate, E_m +80 mV, n = 2 (Sigma Chemical Co.); phenazine ethosulfate, E_m +55 mV, n = 2 (K & K Laboratories, Plainview, N. Y.); duroquinone, E_m +5 mV, n = 2 (Eastman Organic Chemicals, Rochester, N. Y.); pyocyanine, E_m −34 mV, n = 2 (K & K Laboratories, Plainview, N. Y.); 2-hydroxy-1,4-naphthoquinone, E_m −145 mV, n = 2 (Eastman Organic Chemicals, Rochester, N. Y.).

The equipment employed for the determination of the oxidation–reduction potentials of the cytochromes (Dutton, 1970) enabled simultaneous assay of oxidation–reduction potential (platinum and calomel electrodes) and absorbance changes (dual-wavelength spectrophotometer) under strictly anaerobic conditions. The contents (6 ml) of the reaction cuvet (measuring path length 1 cm) were continuously stirred and maintained under an atmosphere of argon (ultra-high purity; O_2 <1 ppm; Matheson Co.). Small additions of reagents were made *via* a 10-μl Hamilton syringe through a septum. Figure 1 shows the behavior of the equipment when containing a suspension of the luminescent microorganism, *Achromobacter fischeri*. Schindler (1964) demonstrated that *A. fischeri* in a salt medium luminesces maximally until the oxygen concentration in the medium is less than 0.3 μM. Following the methods of Schindler with a suspension consuming oxygen at the rate of 7.2 μM/min it was evident that after anaerobiosis was achieved, the level of luminescence was negligible. The addition of 10-μl quantities of aerated medium (0.4 μM oxygen) were rapidly consumed and had no significant effect on the system. Additions of reagents made during experiments were generally less than 2 μl/min and no attempt was made to remove oxygen from reagents added in these small volumes before introduction into the cuvet.

Prior to an experiment, the vessel and contents were thoroughly flushed free of oxygen. Mitochondrial preparations facilitated oxygen removal by respiration of endogenous substrates and of small additions of ascorbate until anaerobiosis was evidenced by a steady oxidation–reduction potential reading of less than +250 mV.

Oxidation–reduction potentials were made more positive with 100 mM potassium ferricyanide and more negative by ascorbate (2–50 mM), NADH (40 mM), or by the action of the endogenous substrates; freshly prepared sodium dithionite in buffer was used to establish potentials lower than −150 mV. It was customary to perform a reductive phase and an oxidative phase of a titration to establish that the oxidation–reduction reactions were reversible and that the system was near equilibrium.

The determinations were carried out at a temperature of 24 ± 2°.

The cytochromes were assayed spectrophotometrically at wavelengths appropriate to their oxidized-minus-reduced spectra in both the α- and γ-band spectral regions. The choice of mediating dyes used for the simultaneous assay was, besides the necessary requirement for electromotive activity, governed by the absence of any significant contributions by the dyes to the absorbance change caused by the oxidation or reduction of the cytochrome. The lack of contribution by the dyes was tested: (a) by titration of the dyes on their own over the same potential range and at the wavelength pairs used to assay the cytochrome, and (b) by increasing the concentration of the dyes by severalfold for the same titration. The latter procedure also gave an indication as to whether the system was close to equilibrium.

The data in this paper are presented graphically as the logarithm of the ratio of oxidized to reduced forms of the cytochromes (abscissa) as a function of potential (ordinate). Oxidation–reduction titrations performed over a wide potential range frequently involved more than one cytochrome with similar spectral characteristics but different midpoint potentials. If, for instance, there are two such cytochromes, a sigmoidal curve is generated and if the midpoints of the two cytochromes are different by 50 mV or more (*cf.* Wilson and Dutton, 1970b), the component cytochromes may be readily resolved arithmetically. The position of the inflection point of the sigmoidal curve with respect to the abscissa indicates the relative contribution of each cytochrome to the total absorbance change at the chosen measuring wavelength.

The reported numbers are the mean of the experimental values plus and minus the range encompassing both the highest and lowest of the values. Limits have not been given to the midpoint potentials of cytochromes a_3 and b_T in the presence of ATP since the values depend on the phosphate potential.

In the case of beef heart mitochondrial preparations, most of the work was done using MAsp since these form the basis for future potentiometric studies of the cytochromes during energy conservation. However, it was found that the results obtained with this preparation, in the presence and absence of uncoupler were essentially the same as those obtained with Esp.

Results and Discussion

Cytochrome c Oxidase. Figure 2 describes the oxidation–reduction potentials of cytochrome oxidase in beef heart

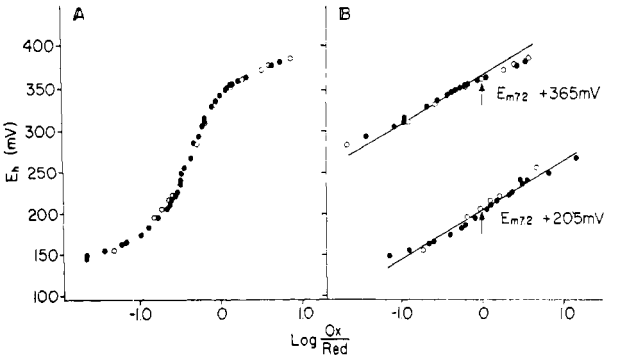

FIGURE 2: The course of oxidation–reduction of cytochromes *a* and a_3 in beef heart submitochondrial particles. MAsp were suspended at 1.9 mg of protein/ml in 0.15 M mannitol, 0.05 M sucrose, and 0.04 M morpholinopropanesulfonate (pH 7.2), in the presence of 17.2 μM S-10. Tetramethylphenylene diamine (20 μM) and approximately 30 μM potassium ferricyanide were added as redox mediators. A reductive phase (●) was effected by small aliquots of ascorbate and an oxidative phase (○) with potassium ferricyanide. The measuring wavelengths were 445 – 455 nm. In part A the logarithm of the ratio of the oxidized to reduced form is for the total cytochrome. Since the titration was not taken further than +400 mV due to a contribution of increased absorbance from ferricyanide as the cytochrome a_3 became oxidized (absorbance decrease), the 100% oxidized absorbance for the titration was estimated, based on cytochrome a_3 being a one-electron carrier. Part B shows the resolution of the sigmoidal curve into its component parts; theoretical $n = 1$ lines are drawn through the points.

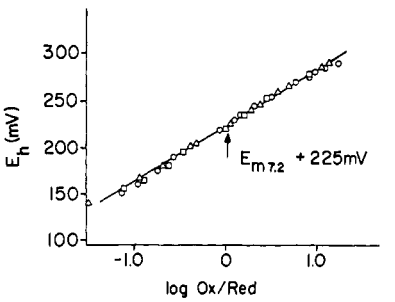

FIGURE 3: The course of oxidation–reduction of cytochromes *c* and c_1 in beef heart submitochondrial particles. MAsp containing approximately equal amounts of cytochrome *c* and c_1 were suspended at 5.2 mg of protein/ml in 0.1 M mannitol, 0.05 M sucrose, and 0.05 M morpholinopropanesulfonate buffer (pH 7.2) in the presence of 17.2 μM S-10. Diaminodurol and phenazine methosulfate were present as redox mediators at the following respective concentrations: 20 μM, 20 μM (○); 35 μM, 35 μM (□); and 50 μM, 35 μM (△). The experimental points were obtained during three reductive titrations from +325 to +110 mV, using NADH as reductant, on the same material; potassium ferricyanide was used to reoxidize the cytochromes. The measuring wavelengths were 550 – 540 nm. A theoretical $n = 1$ line is drawn through the points.

submitochondrial particles. The sigmoidicity (Figure 2A) describes the course of oxidation–reduction of cytochromes *a* and a_3. The reaction of carbon monoxide with cytochrome a_3, but not with *a*, has identified the high-potential component to be cytochrome a_3 (Wilson and Dutton, 1970a); at the measuring wavelengths (445–455 nm), cytochrome a_3 contributes about 60% of the total absorbance change, in agreement with Wohlrab (1970). Resolution of the sigmoidal curve (Figure 2B) produces points which fit closely to two theoretical $n = 1$ curves, showing the $E_{m7.2}$ of cytochromes *a* and a_3 to be +205 ± 10 mV and 365 ± 10 mV, respectively; in rat liver mitochondria the corresponding $E_{m7.2}$ values are +205 ± 10 mV and 390 ± 10 mV, as previously described (Wilson and Dutton, 1970a).

Earlier attempts to determine the midpoint potentials of *a* and a_3 were performed on cytochrome oxidase isolated from beef heart. Comparative techniques were employed, equilibrating the oxidase with ratios of ferri- and ferrocyanide (Ball, 1938) or oxidized and reduced forms of cytochrome *c* (Wainio, 1955; Minnaert, 1965; Tzagaloff and Wharton, 1965). The midpoint potential of "cytochrome oxidase" under these conditions was determined at pH 7.4 to be in the range of +278 to +290 mV. Minnaert (1965), and Tzagaloff and Wharton (1965) reported anomalous *n* values of 0.5. However, the extent of their titrations was limited from +200 to +320 mV, and over this same range in Figure 2A the *n* value is also much less than one, but it represents the transition of the titration between cytochromes *a* and a_3. This could explain the anomaly, but more important, however, T. Tsudzuki and D. F. Wilson (personal communication), using the same technique as described in this paper, recently found that the oxidation–reduction potential properties of isolated cytochrome oxidase are significantly modified as the enzyme is purified.

Tzagaloff and MacLennon (1966) determined the midpoint potential of the copper moiety in isolated beef heart cytochrome oxidase to be $E_{m8.0}$ +284 mV, using a comparative (cytochrome *c*) method. This was recently confirmed by Wharton and Cusanovich (1969). The values obtained, if unmodified in the isolated preparation, would place the copper between the values for cytochrome *a* and a_3 reported in this paper.

Cytochromes c and c_1. Figure 3 describes the oxidation–reduction potentials of endogenous cytochromes $c + c_1$ (molar ratio about 1:1) in beef heart submitochondrial particles. The experimental points fit well with a theoretical $n = 1$ line drawn about a midpoint of +225 mV. The linearity demonstrates the close proximity of the midpoints of the two *c*-type cytochromes in the mitochondrial membrane. The midpoint potentials of cytochromes $c + c_1$ ($n = 1$) in other types of beef heart submitochondrial particles and in intact mitochondria were similar (Table I). Table I also shows that when soluble horse heart cytochrome *c* was bound inside submitochondrial particles (Lee, 1970) to make the ratio of cytochrome $c:c_1$ about 10, the observed midpoint potential is slightly higher than that of the endogenous $c + c_1$ cytochromes; this would imply that the midpoint potential of the cytochrome *c* is marginally higher than that of cytochrome c_1. Soluble horse heart cytochrome *c* alone, when bound inside artificial phospholipid vesicles (Kimelberg and Lee, 1970), also has a midpoint potential close to that observed in the mitochondria.

With the exception of the midpoint potential of soluble beef heart cytochrome *c*, $E_{m7.0}$ +225 mV, reported by Paul (1947), the $E_{m7.0}$ values of both beef and horse heart cytochrome *c* fall in the range +250 mV to + 270 mV (see Clark, 1960). The midpoint potential of the commercial horse heart cytochrome *c* used in the present studies when solved in aqueous media, assumes values, depending on the buffer

TABLE I: Oxidation–Reduction Potentials of *c*-Type Cytochromes in Mitochondrial Preparations.

Cytochrome	Prepn	Medium[a]	pH	E_m (mV)	Absorption[b] Band
c	Soluble cytochrome *c*	MOPS MS-MOPS	7.0 7.2	+283 ± 5	α, γ
c	Soluble cytochrome *c*	Tris-HCl		+283 ± 5	α, γ
c	Soluble cytochrome *c*	Phosphate	7.0	+268 ± 5	γ
c	Soluble cytochrome *c*	Succinate-KCl	7.5	+273 ± 5[c]	γ
c	Soluble cytochrome *c* bound inside phospholipid vesicles	Succinate-KCl	7.5	+230 ± 10[c]	γ
c (*c*:c_1 ~ 10)	Soluble cytochrome *c* bound inside Esp	MS-MOPS	7.2	+235 ± 5	α
c + c_1 (*c*:c_1 ~ 1)	MAsp	MS-MOPS	7.2	+227 ± 5	α
c + c_1	PHM	MS-MOPS	7.2	+233 ± 5	α
c_1	Cytochrome c_1	MOPS	7.0	+225 ± 5	γ

[a] MS-MOPS: 0.1 M mannitol–0.05 sucrose–0.05 M morpholinopropanesulfonate. The other buffers, MOPS, Tris-HCl, and phosphate were all 0.1 M, and succinate-KCl: 0.01 M succinate, 0.15 M KCl. [b] The α band was assayed at 550 − 540 nm using diaminodurol and phenazine methosulfate as mediators (see Figure 3); the γ band was assayed at 416 − 434 nm (Isolated cytochrome c_1 was assayed using 417 − 434 nm.) using 8–20 μM *N,N,N′,N′*-tetramethylphenylenediamine as mediator. [c] Kimelberg and Lee (1970).

used, as much as 60 mV higher than when bound to the mitochondrial particle or phospholipid. Similar behavior of differing midpoint potentials in the free and bound forms of cytochrome b_5 has been reported (Kawai *et al.*, 1963); the $E_{m7.0}$ of cytochrome b_5 is −140 mV in the microsome,

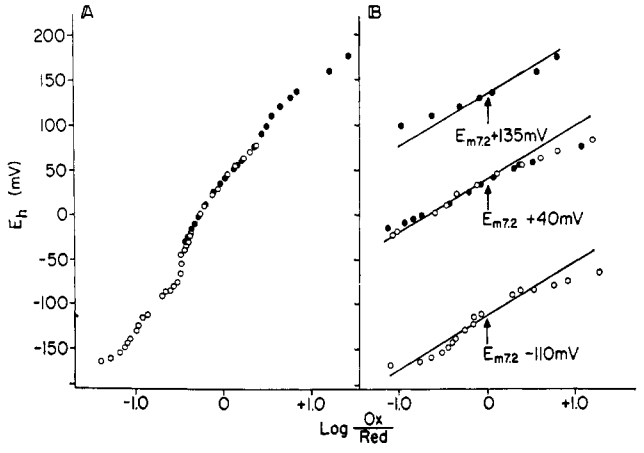

FIGURE 4: The course of oxidation–reduction of components (cytochrome *b*) at 561 − 575 nm in beef heart submitochondrial particles as a function of oxidation–reduction potential. MAsp were suspended at 5.2 mg of protein/ml in 0.15 M mannitol and 0.05 M morpholinopropanesulfonate (pH 7.2); 30 μM diaminodurol, 20 μM phenazine ethosulfate, 50 μM duroquinone, 4 μM pyocyanine, and 25 μM 2-hydroxy-1,4-naphthaquinone were added as redox mediators. A reductive titration (●) using NADH (and lower than −100 mV, sodium dithionite) as reductant, was followed by an oxidative titration (○) using potassium ferricyanide as oxidant. In part A the logarithm of the ratio of the oxidized to reduced is for the total absorbance change from +330 to −180 mV. Part B shows the resolution of the curve into three components; theoretical n = 1 lines are drawn through the points.

+20 mV in aqueous media, and on recombination with microsomal lipid returns to −120 mV.

The changes in midpoint potentials in both cytochromes b_5 and *c* would appear to arise from a preferential binding reaction by the phospholipid for the oxidized form of the cytochrome.

The midpoint potential of the isolated cytochrome c_1 agrees well with the $E_{m7.0}$ +223 mV reported by Green *et al.* (1960). In contrast with cytochrome *c*, the midpoint of cytochrome c_1 is invarient whether in the isolated form or in the mitochondrial membrane. This may be accounted for by the fact that the cytochrome is still in a particulate form and is not solvated in aqueous media; furthermore, the isolated preparation shows no propensity to rebind to mitochondrial membranes (C. P. Lee and H. K. Kimelberg, unpublished observation).

b-Type Cytochromes. Figure 4A shows the absorbance change (562 − 575 nm) as a function of potential in beef heart submitochondrial particles. Resolution of the curve (Figure 4B) in the titration from +330 to −180 mV produces three components: component I, $E_{m7.2}$ +135 mV, which in this case contributes 27% of the total oxidized-minus-reduced absorbance change; component II, $E_{m7.2}$ +40 mV, 47%; and component III, $E_{m7.2}$ −110 mV, 26%. They all appear to be one-electron carriers. A similar titration in the Soret region (430 − 412 nm) produced a similar curve suggesting the presence of the three components in roughly the same proportions observed at the measuring wavelengths in the α-band region. However, there was spectral interference from other cytochromes above +150 mV which made it difficult to resolve component I in the Soret region. An examination of freshly prepared beef heart mitochondria (562–575 nm) showed the three components were present in similar ratios (I, 21%; II, 55%; III, 24%) as observed in the MAsp, indicating that none of the components are artifacts of storage

TABLE II: Oxidation–Reduction Potentials of *b*-Type Cytochromes in Mitochondrial Preparations.

Component	Approximate Contribution to *A* (%)	Wavelengths Measured − Ref (nm)	Prepn	$E_{m7.2}$ (mV)
I	20–35	562–575	BHM, MAsp, and Esp	+125 ± 20
b (II)	45–55			+38 ± 10
III	15–35			−103 ± 15
b	60	561.5–575	PHM[a]	+30 ± 10
b_T	40			−30 ± 10
b	60	561.5–575	PHM + ATP[a]	+30 ± 10
b_T	40			+250
b	50	430–412	RLM[b]	+35 ± 15
b_T	50			−40 ± 20
b	50	430–412	RLM + ATP[b]	+35 ± 10
b_T	50			+245

[a] Chance *et al.* (1970). [b] Wilson and Dutton (1970b).

at −20° or sonication of the intact mitochondria. Esp preparations, which are washed more thoroughly than MAsp particles (*cf.* Löw and Vallin, 1965; Lee and Ernster, 1967), also displays the same ratios of the three components as the parent mitochondria. Furthermore, an Esp preparation derived from mitochondria which were 95% cytochrome *c* depleted by KCl gradient extractions, still contained the three components. This suggests that they are integral parts of the membrane, and is evidence against the possibility that component I is hemoglobin, which has a similar midpoint potential under the experimental conditions. In Table II, the average of five determinations of the midpoint potentials and amounts of the three components in the different beef heart preparations is presented. Accurate midpoint values have not been assigned to components I and III since they lie at the extremities of the titration where the ratio of the oxidized to reduced forms become very sensitive to small errors in absorbance measurements (see Wilson and Dutton, 1970b) and minor contributions from other absorbing species.

Little more can be said about the precise identity of components I and III, but component II is most likely that generally accepted as cytochrome *b* since its $E_{m7.2}$ is of the same order as that found in both pigeon heart and rat liver mitochondria (Table II). The value, however, is 20–40 mV lower than most previously reported values (E_{m7}) in beef heart mitochondrial preparations (Holton and Colpa-Boonstra, 1960; Feldman and Wainio, 1960; Straub and Colpa-Boonstra, 1960; Urban and Klingenberg, 1969). The reason for the discrepancy probably arises from the presence of component I which, since it was not accounted for in the previous determinations, would cause the measured midpoint to be higher. Indeed, analysis of components I and II as one component generates a midpoint potential of about +65 mV, the curve being slightly sigmoidal. An analogous component I is absent in pigeon heart in which the $E_{m7.2}$ of cytochrome *b* is clearly about +30 mV. The titrations (561.5 − 575 nm) of pigeon heart mitochondria from +300 mV to below −200 mV revealed only one other component. This component is considered to be a *b*-type cytochrome with an $E_{m7.2}$ at approximately −30 mV. The differential pH dependencies (D. F. Wilson and P. L. Dutton, unpublished results), of the two *b* cytochromes of pigeon heart mitochondria allowed a clearer potentiometric separation at pH 8.1 than was possible at pH 7.2, as shown in Figure 5; in this case γ band was used, and the curve shows that each cytochrome contributes about 50% to the total absorbance at 430 − 412 nm (*cf.* Table II). Further kinetic studies of these two *b* cytochromes (Chance *et al.*, 1970) have led to a spectral separation showing the reduced-minus-oxidized spectrum of the higher and lower potential *b* cytochromes to be maximal at 560 and 564 nm, respectively. Of the two cytochromes, the lower potential cyto-

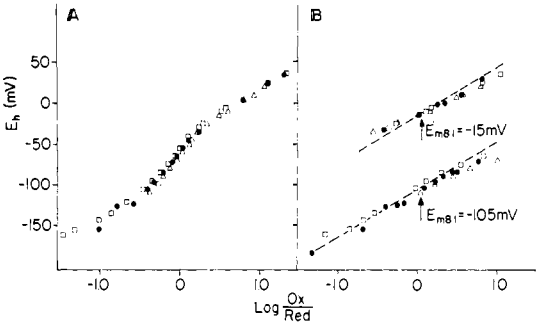

FIGURE 5: The course of oxidation–reduction of *b*-type cytochromes in pigeon heart mitochondria as a function of oxidation–reduction potential. The mitochondria were suspended at 1.9 mg of protein/ml in a 0.05 M sucrose and 0.04 M Tris-HCl buffer (pH 8.1); 30 μM phenazine ethosulfate, 4 μM pyocyanine, 1 μM resoruffin, and 10 μM 2-hydroxy-1,4-naphthaquinone were added as redox mediators. The experimental points △ and □ represent two reductive titrations on the same material, using NADH as reductant and potassium ferricyanide to reoxidize the cytochromes; ● represents a reductive titration from a separate sample of mitochondria under the same conditions. The measuring wavelengths were 430 − 412 nm. In part A the logarithm of the ratio of the oxidized form is for the total cytochrome reacting between +100 and −200 mV. In part B the curve is resolved into two components; theoretical $n = 1$ lines are drawn through the points.

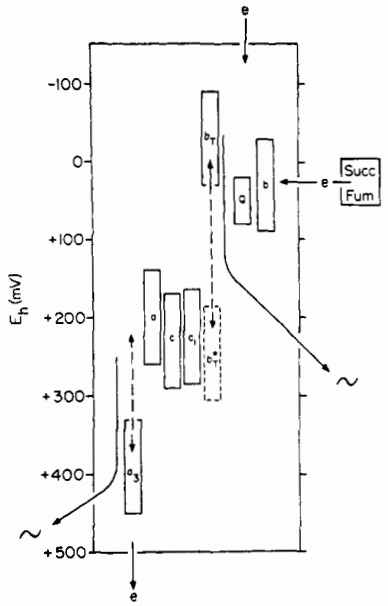

FIGURE 6: Oxidation–reduction potentials of carriers in the mitochondrial respiratory chain. A general description composed of features from rat liver, pigeon heart and beef heart mitochondria. The blocks represent the potentials over which the carriers become 10–90% oxidized or reduced at pH 7.2 in the uncoupled state. The dashed lines indicate changes in midpoint potential of components involved in energy transduction at sites II and III.

chrome *b* in pigeon heart (Chance *et al.*, 1970) and rat liver mitochondria (Wilson and Dutton, 1970b) are considered to be directly involved in energy transduction at site II of the mitochondrial respiratory chain. The energy-transducing cytochrome has been designated cytochrome b_T; its midpoint potential appears to be "energy dependent" and in the presence of ATP assumes a value higher than +240 mV. The $E_{m7.2}$ values, in the presence and absence of ATP are listed in Table II.

Intact beef heart mitochondria also has an energy-dependent *b* cytochrome (Wilson and Dutton, 1970b), but its oxidation–reduction properties remain to be defined. Component III identified in beef heart could be analogous to the b_T cytochromes of pigeon heart and rat liver; however, the midpoint potential of component III seems to be too low to be efficiently reduced by electrons from succinate at the energy-conserving site II.

Figure 6 presents schematically an amalgamation of the common features of the oxidation–reduction potential properties of the cytochromes at pH 7.2 from rat liver, pigeon heart, and beef heart mitochondria, which function for electron transport and energy transduction. It suggests the feasibility of electron flow on purely thermodynamic grounds, and does not propose any mechanisms. The $E_{m7.2}$ of Q (ubiquinone) is taken from the report by Urban and Klignenberg (1969); their determination was done *via* equilibration with succinate:fumarate ratios in beef heart submitochondrial particles using the $E_{m7.0}$ +24 mV at 25° for the succinate–fumarate system in beef heart (Borsook and Schott, 1931).

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