Electron Transport Reversal and Steroid 11β Hydroxylation in Adrenal Cortical Mitochondria*

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ABSTRACT: Evidence for succinate and ascorbate–*N,N,N′,N′*-tetramethyl-*p*-phenylenediamine dihydrochloride (TMPD) mediated electron transport reversal in bovine adrenal cortical mitochondria is presented. Electron spin resonance studies of the kinetics of nonheme iron and fluorescence studies of the kinetics of pyridine nucleotides in conjunction with steroid hydroxylation studies have demonstrated that under suitable conditions the adrenal cortical respiratory chain transmits reducing equivalents to the mitochondrial steroid hydroxylating chain *via* an energy-dependent pyridine nucleotide transhydrogenase. According, a possible role of ascorbic acid in steroid biosynthesis is suggested.

Evidence for reversible electron transport in the respiratory chain was introduced by Chance and Hollunger (1961). They demonstrated that succinate, in the presence of ATP, reduced NAD by a rotenone and a dinitrophenol sensitive pathway. Subsequently, Packer (1962; Packer and Mustafa, 1966) and Tyler and Estabrook (1965) showed that ascorbate plus TMPD[1] also reversed electron transport in an energy-dependent process. The ascorbate–TMPD-supported reduction of NAD *via* reversal is more clearly demonstrated than the reduction by succinate because with succinate, malate generation provides an additional source of reduced pyridine nucleotides.

Harding *et al.* (1968a,b) have demonstrated an ascorbate–TMPD-supported reversal of electron transport in bovine adrenal mitochondria, which is very similar to that reported above in other tissues. This pathway is of particular interest in the adrenal cortex because it can function to provide reducing equivalents to certain mitochondrial steroid hydroxylases (Harding *et al.*, 1968a,b) and because a high concentration of naturally occurring ascorbate exists in this tissue.

Further evidence is presented in this report for the pathway of electron transport reversal and its relationship to steroid hydroxylation in bovine adrenal mitochondria.

Materials and Methods

Bovine adrenal glands were obtained from the Delta Meat Packing Co. in Vernon, Calif. The adrenals were collected on crushed ice within 20 min of death. Cortical tissue was dissected free from the medulla and scraped away from the capsule. The tissue was homogenized in nine volumes of 0.25 M sucrose–1% albumin (pH 7.2) or 0.25 M sucrose–1% albumin–1 mM EDTA (pH 7.2). Mitochondria were separated as described previously (Oldham *et al.*, 1968).

Fluorometric measurements were performed at 23° with the recording fluorometer attachment to the American Instrument Co. dual-wavelength instrument. Oxygen was determined polarographically in the same cuvet. 11β-Hydroxylase activity was assayed as described previously (Oldham *et al.*, 1968). Electron spin resonance studies were done on a Varian E-3 spectrometer with a variable-temperature dewar.

Results

In Figure 1, a typical study of the effect of ascorbate, TMPD, and 11-deoxycortisol on pyridine nucleotide fluorescence, oxygen uptake, and steroid hydroxylation is shown. As previously reported (Harding *et al.*, 1968a,b; Packer and Mustafa, 1966; Tyler and Estabrook, 1965), ascorbate alone does not cause a reduction of pyridine nucleotides, but upon addition of TMPD a rapid reduction of pyridine nucleotide occurs. The addition of 11-deoxycortisol causes a rapid oxidation of the pyridine nucleotides concomitant with its hydroxylation to cortisol. The rate of hydroxylation varied between 12 and 26 mμmoles of cortisol/min per mg of N with the tissue preparation.

The rate of pyridine nucleotide reduction upon addition of TMPD can be seen to be twice as fast as the rate of hydroxylation and yet there is a large drop in the steady state of reduced pyridine nucleotides upon adding 11-deoxycortisol. Also, the rate of oxidation of pyridine nucleotides upon addition of 11-deoxycortisol is twice as fast as the rate of the hydroxylation reaction. This suggests either that the rate of generation of reduced pyridine nucleotides decreases upon addition of 11-deoxycortisol or that reducing equivalents are being utilized for processes other than the hydroxylation of 11-deoxycortisol. Simpson and Estabrook (1969) have shown that NADH oxidase activity in beef adrenal cortex submitochondrial particles is inhibited about 30% by 50 μM deoxycorticosterone. Presumably, a similar inhibition of reversal of electron transport by 11-deoxycortisol occurs in this study.

The insert in Figure 1 shows the relative rate of ascorbate–

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[1] Abbreviations used are: TMPD, *N,N,N,N*-tetramethyl-*p*-phenylenediamine dihydrochloride; PCP, pentachlorophenol.

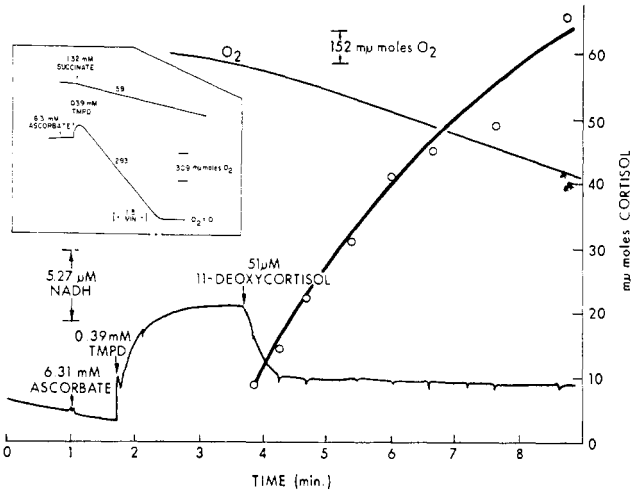


FIGURE 1: Effect of ascorbate–TMPD and 11-deoxycortisol on pyridine nucleotide reduction, oxygen uptake, and steroid hydroxylation. The open circles indicate the amount of cortisol produced at successive time intervals. Each cuvet contained mitochondria equivalent to 0.95 mg of N suspended in a total volume of 3.8 ml containing 13 mM Na_2HPO_4–3 mM KH_2PO_4 buffer (pH 7.2), 6 mM $MgCl_2$, and 96 mM KCl. The pyridine nucleotide calibration was done by adding NADH to a similar mitochondrial suspension without ascorbate–TMPD and 11-deoxycortisol. The rate of steroid hydroxylation was 18 mμmoles of cortisol/min per mg of N.

TABLE I: The Effect of Inhibitors on the Pyridine Nucleotide Fluorescence and Steroid Hydroxylation Supported by Ascorbate–TMPD.[a]

Inhibitor	Concn Producing Half-Maximal Inhibn of Pyridine Nucleotide Fluorescence
Rotenone	1.8 μM
Potassium cyanide	87.0 μM
Pentachlorophenol	3.2 μM
Antimycin A	9.3 μg/ml
Oligomycin	63.0 μg/ml
	Cortisol Formed in 3 min (mμmoles)
None	30
Rotenone, 32 μM	3[b]
Antimycin A, 125 μg	3[b]
Pentachlorophenol, 58.7 μM	0

[a] Reactions were carried out in a total volume of 3 ml in the medium described in Figure 1. Mitochondria equivalent to 1.50 mg of N were used. Ascorbate (6.31 mM) and TMPD (0.39 mM) were used. 11-Deoxycortisol conversion into cortisol was estimated as described previously (Oldham *et al.*, 1968). [b] The first sample was removed after a few seconds and showed 3 mμmoles of cortisol. The rate was zero. The amount of cortisol formed in 3 min without ascorbate–TMPD or inhibitors was 3 mμmoles. 11-Deoxycortisol (51 μM) was used.

TMPD and succinate oxidase. The actual rate varied from preparation to preparation depending upon the degree of respiratory control.

The capacity of adrenal mitochondria to perform this apparent reversal of electron transport is dependent upon addition of 1% albumin to the 0.25 M sucrose homogenizing medium. EDTA (1 mM) added to this medium was necessary for the exogenous ATP-supported reversal. However, EDTA appeared to show no effect on the other reactions studied. The addition or elimination of $MgCl_2$ from the isotonic medium in which the mitochondria were diluted for study produced no detectable change. However, the elimination of KCl from this medium caused an approximate 50% decrease in reduced pyridine nucleotides.

The effect of varying concentrations of ascorbate and TMPD on the reduction of pyridine nucleotides was studied. Using mitochondria equivalent to 1.1 mg of N, the optimal concentrations of ascorbate and TMPD were 6.31 and 0.39 mM, respectively.

To further characterize the pathway of electron transport, the effect of potassium cyanide, antimycin A, rotenone, oligomycin, and PCP on the reduction of pyridine nucleotides was studied by performing inhibitor *vs.* pyridine nucleotide reduction titration curves. The concentrations of inhibitor achieving half-maximal inhibition are summarized in Table I. Ninety-eight per cent maximal inhibition was produced by 180 μM potassium cyanide, 50 μg of antimycin A, 8.0 μM rotenone, and 100% inhibition by 4.0 μM PCP. Oligomycin achieved only 56% inhibition at 250 μg in 3-ml total volume. Table I also shows the effect of maximally inhibitory concentrations of rotenone, antimycin A, and PCP on the hydroxylation of 11-deoxycortisol. A very small amount of hydroxylation occurs in the presence of potassium cyanide, antimycin A, and rotenone. The uncoupler, PCP, completely inhibited hydroxylation activity, however. These results are consistent with the effects of these inhibitors on pyridine nucleotide reduction. In the absence of added electron donor a low level of steroid hydroxylation is seen comparable to the rates seen in the presence of the above inhibitors. Presumably, this endogenous donor is sensitive to PCP since this agent completely inhibited hydroxylation. Assuming that the endogenous donor was malate this effect of the uncoupler could perhaps be attributed to an inhibition of malic enzyme analogous to the DNP inhibition of this enzyme reported by Simpson and Estabrook (1969). However, oligomycin studies to be presented below suggest that the effect of PCP is more likely related to an inhibition of an energy-dependent process.

The effect of ADP on the steady state reduction level of pyridine nucleotides was studied. Using mitochondria equivalent to 1.2 mg of N, the concentration of ADP required for maximal oxidation of the pyridine nucleotides was 0.98 mM. At this concentration, phosphorylation of ADP apparently depletes the high-energy intermediates to a level that will no longer support reversal of electron transport. Chance and Hollunger (1961) and Packer and Mustafa (1966) have demonstrated a similar phenomenon. Oligomycin (4.2μg/mg of N) completely prevents the ADP-induced oxidation. The effect of ADP on steroid hydroxylation is shown in Figure 2. Addition of 0.55 mM ADP as well as 11-deoxycortisol does increase the rapid oxidation of the pyridine nucleo-

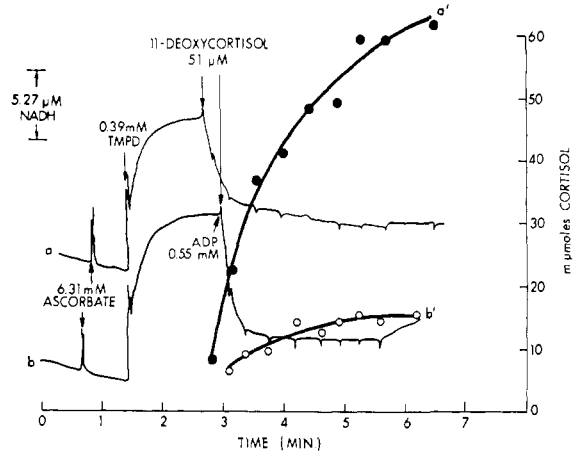


FIGURE 2: The effect of ADP on pyridine nucleotide fluorescence and on steroid hydroxylation. Line a is the fluorescence change and line a′ the amount of cortisol formation without ADP. Line b shows fluorescence and line b′ the amount of cortisol formation in the presence of 0.55 mM ADP. Mitochondria equivalent to 1.20 mg of N were suspended in a total volume of 3.8 ml in the medium described in Figure 1. The rates of steroid hydroxylation are a′ = 26 mμmoles of cortisol/min per mg of N and b′ = 3 mμmoles of cortisol/min per mg of N.

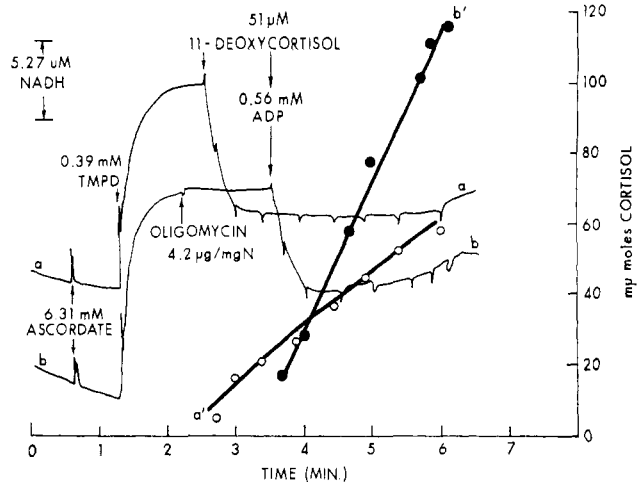


FIGURE 3: The effect of oligomycin and ADP on ascorbate–TMPD pyridine nucleotide fluorescence and steroid hydroxylation. Lines a and b show fluorescence changes, a without oligomycin, b with oligomycin. Lines a′ and b′ show the amount of cortisol formation. The mitochondrial concentration and suspending medium were identical with Figure 2. The rates of steroid hydroxylation were a′ = 13 mμmoles of cortisol/min per mg of N and b′ = 38 mμmoles of cortisol/min per mg of N.

tides, but achieves the same oxidized steady state. Comparison of lines a′ (cortisol formation in the absence of ADP) and b′ (cortisol formation in the presence of ADP) shows, however, that it almost completely inhibits hydroxylation. The effect of 4.2 μg/mg of N of oligomycin on the ADP inhibition is shown in Figure 3. Oligomycin not only completely abolishes the ADP inhibition (line b′) but, as shown by comparison with line a′, stimulates the rate of hydroxylation almost threefold. The stimulation of steroid hydroxylation with low concentrations of oligomycin may be the result of stabilization of a high-energy intermediate of oxidative phosphorylation, as suggested by Danielson and Ernster (1963), Lee and Ernster (1968b), and Oldham *et al.* (1968) to explain the stimulation of energy-dependent pyridine nucleotide transhydrogenase. The increased steady-state reduction level of pyridine nucleotides in the presence of low oligomycin concentrations despite a stimulation of hydroxylation activity suggests that in addition to electron transport reversal, the pyridine nucleotide transhydrogenase is also stimulated.

Chance and Hollunger (1961) showed that exogenous ATP supported succinate reversal of electron transport in a sodium sulfide blocked respiratory chain. We have shown a similar phenomenon in the ascorbate–TMPD-supported system in KCN-inhibited adrenal mitochondria. No effect of exogenous ATP on the noninhibited system was found indicating that the availability of high-energy intermediates was not rate limiting. In accordance with the observations of Chance and Hollunger, we found that ADP, phosphate, and magnesium inhibited this ATP-supported reversal. Therefore, the mannitol–sucrose–Tris medium described by these investigators, with the addition of 96 mM KCl, was employed. The effect of varying concentrations of ATP on the rate and maximal reduction of pyridine nucleotides in the KCN, ascorbate–TMPD-treated mitochondrial preparation was studied. In the presence of mitochondria equivalent to 1.6 mg of N, 1.24 mM ATP gave the maximal amount of reduced pyridine nucleotides.

A comparison of pyridine nucleotide reduction and steroid hydroxylation in the isotonic phosphate–magnesium–KCl buffer used in the earlier studies *vs.* the mannitol–sucrose–

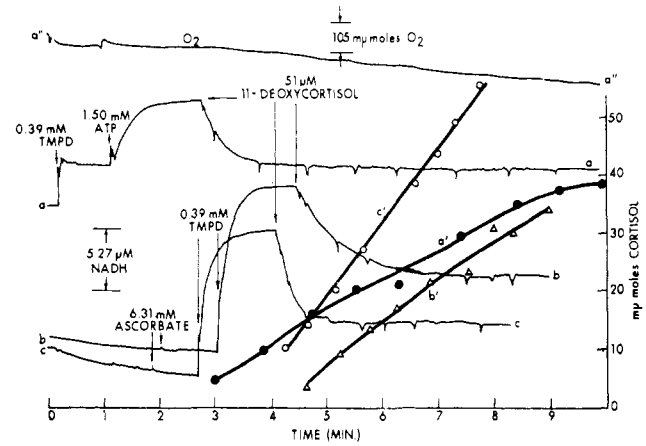


FIGURE 4: The effect of exogenous ATP on pyridine nucleotide fluorescence and steroid hydroxylation in the presence of KCN, ascorbate–TMPD, and 11-deoxycortisol. Line a shows fluorescence, line a′ shows steroid hydroxylation, and line a″ shows oxygen uptake in the presence of 0.71 mM KCN and 6.31 mM ascorbate. Mitochondria equivalent to 1.1 mg of N were suspended in a total volume of 3.8 ml containing 0.23 M mannitol, 0.07 M sucrose, 0.02 M Tris, and 96 mM KCl (pH 7.2). Lines b, c show fluorescence and lines b′, c′ show steroid hydroxylation without KCN. The mitochondrial concentrations were identical with line a. The suspending medium in line b is the same as line a and that of line c is the phosphate medium described in Figure 1. The rates of steroid hydroxylation are a″ = 5 mμmoles of cortisol/min per mg of N, b″ = 6 mμmoles of cortisol/min per mg of N, and c′ = 12 mμmoles of cortisol/min per mg of N.

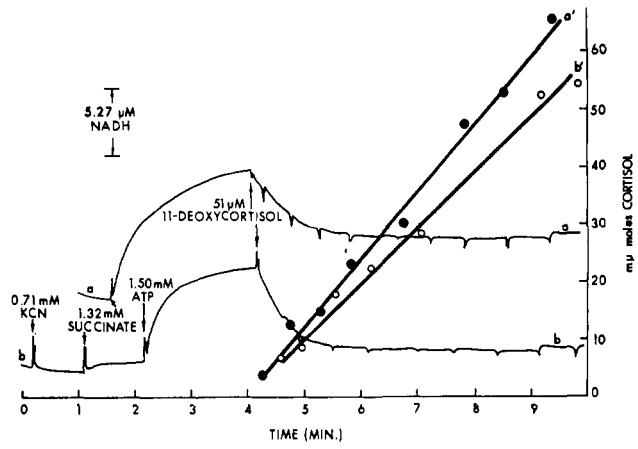


FIGURE 5: The effect of exogenous ATP and succinate on pyridine nucleotide fluorescence and steroid hydroxylation in the presence of KCN. Line a shows fluorescence and line a′ shows steroid hydroxylation without KCN and ATP. Line b shows fluorescence and line b′ shows steroid hydroxylation in the presence of KCN and ATP. Mitochondria equivalent to 1.1 mg of N were suspended in a total volume of 3.8 ml in the mannitol medium described in Figure 7. The rates of steroid hydroxylation are a′ = 11 mμmoles of cortisol/min per mg of N and b′ = 9 mμmoles of cortisol/min per mg of N.

Tris–KCl solution is shown in Figure 4 (b,b′,c,c′). In phosphate buffer (4c) the degree of reduction is slightly less than that seen in the mannitol solution (4b). But the rate of hydroxylation is 12 mμmoles of cortisol/min per mg of N (4c′) *vs.* 6 mμmoles/min per mg of N in the mannitol buffer (4b′). This difference is associated with a slightly greater steroid substrate induced oxidative shift in the pyridine nucleotides in the phosphate medium. ATP induced reduction of pyridine nucleotides, support of hydroxylation, and oxygen uptake are shown in Figure 4a,a′,a″. The steady-state level of reduction of the nucleotides is significantly smaller than that seen in the noninhibited respiring systems in Figure 4b,c. Apparently, exogenous ATP is not as efficient as internally generated high energy intermediates in supporting the reversal, or the transport of added ATP into the mitochondrion is rate limiting. Nevertheless, the rate of hydroxylation, 5 mμmoles of cortisol/min per mg of N, is comparable to that observed in the noninhibited system (Figure 4b). Several explanations for this anomaly might be suggested: pyridine nucleotide reduction may not be rate limiting in the mannitol–sucrose–Tris–KCl medium; exogenous ATP may allow the reduction of only a limited pool of the total pyridine nucleotides; or it may facilitate a by-pass of reducing equivalents to the hydroxylating system.

The effect of rotenone, antimycin A, PCP, and oligomycin on steroid hydroxylation in the cyanide-treated ascorbate–TMPD supported system is shown in Table II. PCP and oligomycin completely block steroid hydroxylation, while rotenone and antimycin A permit only a very low and transient activity. This activity is comparable to that seen without the addition of electron donor and as in the case of the respiring system is thought to arise from the presence of an endogenous donor not inhibitable by rotenone and antimycin A, but dependent upon high-energy intermediates derived from oxidative phosphorylation.

The role of succinate-supported electron transport reversal in steroid hydroxylation is controversial. Cammer and Estabrook (1966) reported that ADP inhibited steroid hydroxylation in a succinate-supported system suggesting that an energy-dependent succinate-supported reversal functioned in this system. Cammer and Estabrook (1967) later reported that ADP had no effect on succinate-supported hydroxylation. Harding *et al.* showed that amytal (1968a) and rotenone (1968b) partially inhibited pyridine nucleotide reduction by succinate. They also pointed out that under noninhibitory conditions the amount of malate generated by saturating concentrations of succinate was approximately 0.092 mM/min. The rate of hydroxylation which this concentration of malate could support would have been approximately 20 mμmoles/min, significantly less than the rate of 53 mμmoles/min actually supported by succinate.

To elucidate further the pathway for succinate-supported hydroxylation, studies were done similar to those with the ascorbate–TMPD–KCN-treated system. The effect of succinate on pyridine nucleotide reduction and on steroid hydroxylation is shown in Figure 5. Figure 5a,a′ shows the effect of succinate alone and 5b,b′ the effect of succinate plus ATP in the KCN-treated system. Although the rate and extent of pyridine nucleotide reduction are different, the rates of hydroxylation in these two systems (9–11 mμmoles of cortisol/min per mg of N) are not. No hydroxylation lag is observed in either system. The inhibitor sensitivity of ATP-supported hydroxylation in this system is shown in Table II. PCP and oligomycin completely inhibit pyridine nucleotide reduction and steroid hydroxylation. Rotenone and antimycin A permit only a small and transient rate

TABLE II: The Effect of Inhibitors on the Exogenous ATP-Supported Electron Transport Reversal and Steroid Hydroxylation.[a]

	Rate of Cortisol Formation/mg of N (mμmoles/min per mg of N)	
	(ATP + KCN) Succinate	(ATP + KCN) Ascorbate–TMPD
None	9.08	4.55
Antimycin A, 250 μg		0.95[b]
Rotenone, 32 μM	0.95[b]	1.09[b]
Pentachlorophenol, 58.7 μM	0	0
Oligomycin, 5 μg	0	0

[a] Total incubation volume was 3.8 ml using the medium described in Figure 4. Tissue equivalent to 1.1 mg of N was used. 11-Deoxycortisol conversion into cortisol was estimated as described previously (Oldham *et al.*, 1968). Ascorbate (6.31 mM), TMPD (0.39 mM), succinate (1.32 mM), 11-deoxycortisol (51 μM), KCN (0.71 mM), and ATP (1.50 mM) were used. [b] The amount of cortisol formed in 3 min without electron donor or inhibitors was approximately 1.0 mμmoles/min per mg of N. After this time no additional conversion occurred. Rotenone and antimycin A in the presence of electron donor permitted a similar conversion.

of hydroxylation similar to that observed with ascorbate–TMPD. Again, this conversion is comparable to that seen without the addition of electron donor presumably arising from an endogenous donor not inhibitable by rotenone but sensitive to uncoupler and oligomycin.

These succinate studies demonstrate that ATP-dependent succinate-supported reversal of electron transport can occur in cyanide-treated bovine adrenocortical mitochondria. Further, because of the rapid rate of pyridine nucleotide reduction and the absence of a lag period in steroid hydroxylation, it appears unlikely that succinate provides reducing equivalents *via* the formation of malate.

In an attempt to characterize further the relationship of the hydroxylating pathway to the respiratory chain, the kinetics of redox shifts in nonheme iron were compared with kinetics of pyridine nucleotide redox changes. Reduced adrenodoxin of the hydroxylating pathway and reduced nonheme iron of the respiratory chain show prominent electron spin resonance signals at $g = 1.94$ and 2.01 (Watari and Kimura, 1966; Beinert and Sands, 1960). Changes in the nonheme iron carrier of the hydroxylating pathway cannot be distinguished from the nonheme iron carriers of the respiratory pathway (Beinert and Sands, 1960). Figure 6 (insert) shows the kinetics of pyridine nucleotide fluorescence in response to addition of several agents. At the times indicated, samples were removed, placed into quartz electron spin resonance tubes, and frozen in liquid nitrogen. Figure 6 also shows the first derivative electron spin resonance curves obtained from the samples removed at the times indicated. The maximally reduced state of nonheme iron after dithionite treatment is shown in Figure 6D. The fully oxidized mitochondrial preparation is shown without any additions in 6A. This nonheme iron signal is very small, indicating almost complete oxidation. After the addition of ascorbate–TMPD a marked increase in the signal is seen in 6B. This increase, however, is only 76% of that seen with dithionite (6D). This difference may reflect the fact that all of the nonheme iron is not in the ascorbate–TMPD pathway or that in the presence of oxygen complete reduction cannot be achieved. Upon addition of 11-deoxycortisol in 6C, a decrease in the signal to its initial level is seen. Complete return to the oxidized spectrum indicates that all of the nonheme iron that is reduced by ascorbate–TMPD (76% of the total) presumably including nonheme iron of the respiratory chain and adrenodoxin of the hydroxylation pathway is affected by the oxidative drain imposed by steroid hydroxylation.

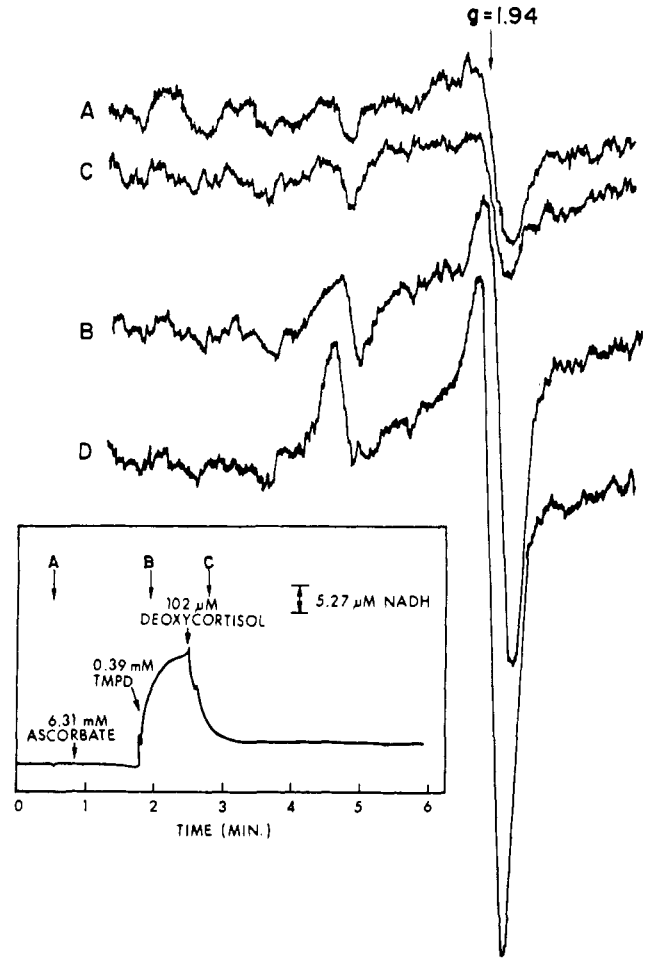
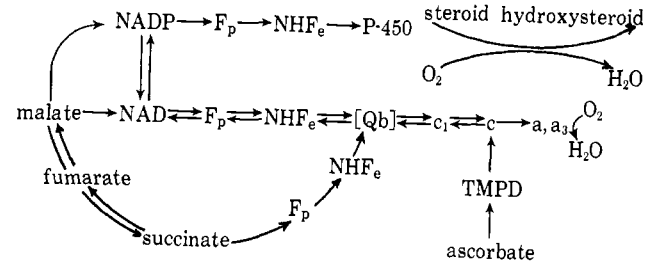


FIGURE 6: The effect of ascorbate–TMPD and 11-deoxycortisol on pyridine nucleotide fluorescence and nonheme iron. The insert shows pyridine nucleotide changes and at the times indicated samples were removed for electron spin resonance spectroscopy. Sample d was removed after the reaction was monitored and sodium dithionite was added to the cuvet. The first derivative curves are shown in lines a–d. Mitochondria equivalent to 2.1 mg of N were suspended in a 3.8-ml total volume in the medium described in Figure 1.



Discussion

These studies of redox changes in pyridine nucleotides and nonheme iron clearly demonstrate that a reversal of electron transport similar to that previously described in other tissues by Chance and Hollunger (1961), Packer and Mustafa (1966), and Tyler and Estabrook (1965) can occur in the adrenal cortex. Of special interest to us was the fact that this reversal of electron transport in the adrenal mitochondria could support steroid hydroxylation. This offers further support for the following relationship of the respiratory chain and the energy-linked pyridine nucleotide transhydrogenase to the hydroxylating system, as originally suggested by Harding and collaborators (1965, 1966).

Simpson and Estabrook (1969) have disputed the significance of the energy-linked transhydrogenase in beef adrenal cortex when malate and succinate are used as substrates. They have proposed that the mitochondrial transhydrogenase functions in a nonenergy-linked manner operating unidirectionally from NADPH–NADH. This proposal was suggested on the basis of pyruvate accumulation in the presence of arsenite and malate in the absence of steroid substrate which did not occur in the presence of rotenone. This sug-

gested a rotenone-sensitive reoxidation of NADPH *via* the pyridine nucleotide transhydrogenase. They also noted that the amount of pyruvate generated was enough to provide almost all the NADPH required to maintain the observed hydroxylation rate. However, in a similar study where succinate was used as a source of reducing equivalents no accumulation of pyruvate was noted. Further, the rotenone-sensitive reoxidation of NADPH is open to question since the evidence for the increased rate of pyruvate generation in the absence of rotenone is inconclusive. The second point, that the amount of pyruvate generated was enough to provide almost all the NADPH required to maintain the hydroxylation rate, does not prove that the energy-linked transhydrogenase is nonfunctional. From our foregoing studies, little doubt exists that reducing equivalents from ascorbate–TMPD and from succinate in the presence of cyanide are transported exclusively by a reversal of electron transport coupled to the pyridine nucleotide transhydrogenase. Although it is clear that reversal under these conditions does not occur *in vivo*, the possibility exists that reversal could occur under physiological conditions.

Cammer and Estabrook (1967) have suggested that there is a deficiency or alteration in the terminal steps of energy transfer for ATP formation in beef adrenals. One of the points of evidence used for this conclusion was that ADP could not compete with hydroxylation in a succinate-supported system. Since succinate oxidation leads to malate production and addition of ADP by mediating a state 4–3 transition would accelerate this production, it might be expected that ADP would inhibit only the initial rate of hydroxylation which might not be apparent in the overall rate. In the ascorbate–TMPD system ADP showed a clear oligomycin-sensitive inhibition of pyridine nucleotide reduction and hydroxylation, indicating normally functioning terminal steps of energy transfer for ATP formation. As suggested by the studies by Lee and Ernster (1966, 1968a) on beef heart submitochondrial particles, this is probably the result of competition between oxidative phosphorylation and the energy-linked transhydrogenase. The differences between Cammer and Estabrook's and our own results regarding ADP inhibition of steroid hydroxylation may be due to differential conditions affecting the energy-yielding and energy-consuming process of the mitochondrial preparation.

The role of adrenal cortical ascorbic acid continues to remain elusive. The observation by Sayers *et al.* (1944) that adrenal ascorbic acid decreases in response to stress or ACTH stimulation has prompted many investigations attempting to understand its function in adrenal metabolism and corticosteroidogenesis. Hayano *et al.* (1956) reported that 10 mM ascorbic acid inhibited 11β hydroxylation about 65%. Kersten *et al.* (1958) reported the presence of NADH oxidase system in adrenals which employs ascorbic acid–dehydroascorbic acid as a redox couple to provide reducing equivalents for steroid hydroxylation. A more recent study by De Nicola *et al.* (1968) suggests that ACTH inhibits ascorbic acid transport into the adrenal cell, thus implying again that ascorbic acid normally plays an inhibitory role in steroidogenesis. Although a naturally occurring redox couple permitting ascorbate entry into the electron transport system for hydroxylation has not been demonstrated to date, these studies which show that ascorbate under certain conditions can provide the energy to support 11β hydroxylation at a rate comparable to that supported by Krebs cycle intermediates provide additional interest in the possible existence of such a couple.

Acknowledgments

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