Pyridine Nucleotide Transhydrogenase

VIII. PROPERTIES OF THE TRANSHYDROGENASE REACTIONS OF AN ENZYME COMPLEX ISOLATED FROM BEEF HEART MITOCHONDRIA*

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Extensive studies in this and other laboratories have demonstrated the occurrence of pyridine nucleotide transhydrogenase activity in a variety of animal mitochondria (1-6), bacterial extracts (7), mitochondrial particles (8-10), and plant tissues (11). With the introduction of the pyridine nucleotide analogues (5) the study of the true transhydrogenase reaction was greatly facilitated. This reaction, given in Equations 1 and 1a, will be referred to as the DD-transhydrogenase.

\[
\text{TPNH} + \text{DPN} \rightarrow \text{TPN} + \text{DPNH} \quad (1)
\]

\[
\text{TPNH} + 3\text{-acetylpyridine-DPN} \rightarrow \text{TPN} + 3\text{-acetylpyridine-DPNH} \quad (1a)
\]

In addition, the homologous transhydrogenase or pyridine nucleotide exchange reaction, Equations 2 and 2a, will be referred to as the TD-transhydrogenase reaction.

\[
\text{DPNH} + \text{DPN} \rightarrow \text{DPN} + \text{DPNH} \quad (2)
\]

\[
\text{DPNH} + 3\text{-acetylpyridine-DPN} \rightarrow \text{DPN} + 3\text{-acetylpyridine-DPNH} \quad (2a)
\]

In view of the probable fundamental role of these enzymes in the oxidation of reduced pyridine nucleotides, particularly reduced triphosphopyridine nucleotide, and the current controversial views concerning the nature of their importance (12) and mechanism of action (13, 14), it was of importance to examine the purification and properties of these enzymes in detail. In this investigation, the isolation and properties of an enzyme complex with high transhydrogenase activity from beef heart mitochondria will be described. In addition, information will be presented on the relationship between the transhydrogenase reactions and the enzymes associated with the terminal electron transport system of heart mitochondria.

EXPERIMENTAL PROCEDURE

Cofactors and Other Substances—The 3-acetylpyridine analogue of DPN was prepared according to the method of Kaplan and Ciotti (15). The thionicotinamide analogue of DPN was prepared according to the method of Anderson et al. (16). The other DPN analogues utilized in these studies were prepared by the general procedures described by Kaplan and Stolzenbach (17).

The abbreviations used are: EDTA, ethylenediaminetetraacetic acid.

1 The abbreviation used is: EDTA, ethylenediaminetetraacetic acid.
duction of the dye was followed at 600 nm. Mercaptoethanol must be removed from the enzyme by dialysis before the diaphorase assay is carried out.

_Cytochrome c Reductases—DPN and TPN cytochrome c reductase was determined by the method of Lehman and Nason (18) modified for a final volume of 1 ml. The reaction mixture consisted of 100 μmoles of potassium phosphate buffer, pH 7.5, 1 μmole of KCN, 0.1 ml of 2% aqueous cytochrome c, and 1 μmole of the appropriate reduced pyridine nucleotide. The reduction of cytochrome c was followed at 550 nm._

_DPNH and TPNH Oxidases—The reaction mixture (final volume, 1 ml) consisted of 100 μmoles of phosphate buffer, pH 7.5, and 0.2 μmole of DPNH (or TPNH), with or without the addition of 0.04 ml of 2% aqueous solution of cytochrome c. The disappearance of DPNH or TPNH was measured at 340 nm._

_Lipoic Dehydrogenase—The oxidation of DPNH by lipoamide was determined in a reaction mixture which consisted of 100 μmoles of phosphate buffer, pH 7.5, 0.2 μmole of DPNH, and 1 μmole of DL-lipoamide in a final volume of 1 ml._

_Protein Determinations—Protein was estimated either by the biuret method (19), by the method of Lowry et al. (20), or by spectrophotometric measurement at 260 and 280 μm (21)._!

**RESULTS**

_Purification of Enzymes—All steps in the purification procedure, outlined in Table I, were carried out at 0 to 5°._

_Beef hearts obtained from freshly killed animals were quartered, trimmed, and passed through an electric meat grinder. Ground heart muscle (800 g) was homogenized for 3 minutes in a large Waring Blender with 2400 ml of cold 0.25 M sucrose containing 0.01 M K₂HPO₄, pH 8.6, and 0.005 M EDTA. The homogenate was adjusted to pH 8.5 with 6 N KOH and immediately centrifuged for 10 minutes at 1000 × g in the bucket head of an International centrifuge. The supernatant suspension was carefully decanted through cheesecloth and diluted with 5 liters of 0.9% KCl containing 0.005 M EDTA, pH 7.6. The mitochondria were sedimented by passing the suspension through a Serval centrifuge at 300 to 350 ml per minute (55,000 r.p.m.). The mitochondrial paste was resuspended by brief homogenization in 5 volumes of 0.25 M sucrose containing 0.01 M KH₂PO₄, pH 7, and 0.005 M EDTA, and was stored overnight at 0°._

_All reagents used in the subsequent purification of the enzymes were made up in glass-distilled or deionized water and routinely contained 5 × 10⁻⁴ M EDTA and 1 × 10⁻⁴ M mercaptoethanol, except for elution of Ca₃PO₄ gel when the EDTA was omitted. Ammonium sulfate was recrystallized from a weakly basic solution of 0.05 M EDTA._

The mitochondria were resedimented by centrifugation at maximal speed in a Serval centrifuge and resuspended in 2 volumes of 0.05 M potassium phosphate buffer, pH 7. A chilled solution of digitonin, prepared by heating in a boiling water bath a suspension of digitonin in 0.05 M potassium phosphate, pH 7, was added to the mitochondrial suspension. The final concentration of digitonin added to the mitochondria was 2 mg per mg of mitochondrial protein. After incubation in an ice bath for 2 hours with occasional stirring, the suspension was centrifuged for 1 hour at 30,000 r.p.m. in the No. 30 head of the Spinco centrifuge and the gelatinous residue discarded.

Absolute ethanol, prechilled to −70°, was added dropwise to the digitonin extract while the temperature was maintained just above the freezing point. At a 25% ethanol concentration and a solution temperature of −12°, the resulting precipitate was immediately centrifuged at maximal speed in a Serval centrifuge maintained at −15 to −20°. The sticky, gelatinous, red precipitate was redissolved in 0.05 M potassium phosphate buffer, pH 7.

The deep red solution was treated serially with small portions of calcium phosphate gel (29), approximately 0.1 mg of gel per mg of protein. After discarding the first two gels, the remaining gels, which had removed over 90% of the transhydrogenase activity, were pooled and exhaustively washed (five times) with about 30-ml portions of 0.1 M potassium phosphate buffer, pH 8, containing 1 mg of gel per mg of protein. A large amount of colored material, protein, and about 10% of the TD-transhydrogenase activity were removed in this manner (Fraction 4). The transhydrogenase activity was eluted by resuspending the gel in 20 ml of 40% saturated ammonium sulfate dissolved in 0.1 M acetate buffer, pH 4.6. After standing for 1 hour with occasional stirring, the gel suspension was centrifuged and the eluate recovered. Usually two elutions were necessary to recover all the transhydrogenase activity (Fraction 4a).

Solid ammonium sulfate was added very slowly to the clear yellow supernatant solution (Fraction 4a) to approximately 50% saturation. After standing for 30 minutes the suspension was centrifuged and the colorless supernatant solution was discarded. The brownish precipitate was resuspended in 0.05 M potassium phosphate buffer, pH 7. The pale yellow solution was dialyzed for 5 hours against 10 volumes of the resuspending buffer and frozen (Fraction 5).

_Fraction 5 contained the highest specific activity of both_
transhydrogenase enzymes, usually representing a 10- to 15-fold purification and approximately 15% recovery of the original activity. The removal of approximately 30% of the total protein without a significant change in the specific activity of the transhydrogenase reaction during the preparation of the 100,000 × g digitonin extract (Fraction 2) is probably an indication of the complex particulate nature of this system. The chief advantage of the ethanol precipitation step (Fraction 3) is the stabilization of the TD activity resulting from removal of the digitonin from the preparation. However, because of the marked lability of the TD-transhydrogenase, the entire purification of the extract was generally carried out without interruption. Further attempts to purify the enzymes resulted in a rapid loss of the TD-transhydrogenase activity.

As was previously reported (1), treating the mitochondria with 10% ethanol at 40° or conversion to an acetone powder resulted in a complete loss of the TD activity. Similar loss of activity was obtained after treating the mitochondria with n-butanol or tert-amyl alcohol. Although treatment with cholate or deoxycholate solubilized the mitochondria, only a small fraction of the transhydrogenase activity could be recovered in the supernatant solution after high speed centrifugation.

Various ionic and nonionic detergents yielded similar results. However, in all previously mentioned instances, the DD-transhydrogenase was easily extracted from the mitochondria. The most useful method of extracting the DD-transhydrogenase activity was to extract the mitochondria with 1% ammonium sulfate (23); however, this procedure resulted in a complete loss of the TD-transhydrogenase activity.

Stability of Enzymes—The final enzyme preparation was stable for several months upon storage at -15°. At 5°, the TD-transhydrogenase lost approximately 10% of its activity per day, whereas no loss of DD-transhydrogenase activity was evident for 5 days. Repeated freezing and thawing resulted in about 10 to 15% loss of TD-transhydrogenase activity per cycle with little or no effect on the DD enzyme. Fig. 1 illustrates the loss of both transhydrogenase reactions of Fraction 5 upon exposure to a temperature of 46°. Thus, despite the relative stability of the DD-transhydrogenase to extraction and manipulation, it is observed to decay in this fraction at essentially the same rate as the more labile TD enzyme. Under the same conditions, the DD activity in Fraction 4 is quite stable. The TD activity in Fraction 4 decayed completely within a few days at -15°. Exposure of Fraction 5 to a temperature of 65° for 5 minutes resulted in a decrease in activity of 100 and 85% for the TD-transhydrogenase and DD-transhydrogenase, respectively.

The DD-transhydrogenase was quite stable to dialysis against phosphate buffer, whereas the TD-transhydrogenase lost approximately 90% of its activity within 12 hours unless the dialysis tubing was previously boiled in dilute alkaline EDTA and exhaustively washed with deionized water. Under these conditions, dialysis could be carried out for 5 or 6 hours with little loss in activity. Extensive dialysis, for 8 hours or more, resulted in the loss of about 50% of the TD-transhydrogenase and the sporadic formation of an insoluble precipitate containing variable amounts of both transhydrogenase activities.

Further attempts to purify the transhydrogenase enzymes utilizing ethanol, n-butanol, or tert-amyl alcohol resulted in the

Fig. 1. Inactivation of transhydrogenase reactions and activation of diaphorase reaction by mild heat. At specified times aliquots of the purified enzyme (Fraction 5), prior incubation at 46°, were removed and assayed for TD-transhydrogenase, DD-transhydrogenase and DPNH-diaphorase by the standard techniques described under "Experimental Procedure."
Table III summarizes the effect of antimycin A on the various reactions observed in the beef heart transhydrogenase system. This antibiotic is strongly inhibitory only with the DPNH-cytochrome c reductase reaction. However, somewhat higher concentrations of the inhibitor than reported elsewhere (18) appear to be required for complete inhibition. The lack of antimycin A sensitivity of the TPNH-cytochrome c reductase reaction has been reported recently for a similar enzyme obtained from beef heart muscle by Lang and Nason (26).

Table IV summarizes the effect of Amytal on the various reactions in this system. The TPNH-cytochrome c reductase enzyme exhibits a partial sensitivity to Amytal. As with antimycin A, the DPNH-cytochrome c reaction is strongly inhibited by Amytal, whereas the transhydrogenase reactions are not affected.

Various attempts to remove the oxidase and cytochrome c reductase activities from the transhydrogenase activities were unsuccessful. Centrifugation at 100,000 × g for 4 hours resulted in the sedimentation of the entire complex of enzymes without significant changes in the ratios of the various activities. Although the very small amount of transhydrogenase remaining in the supernatant fraction had a higher ratio of TD- to DD-transhydrogenase, DPNH-cytochrome c reductase activity could not be detected with high enzyme concentration and added cytochrome c. With the addition of equal amounts of DPN, a significant rate of TPNH oxidation was observed which was stimulated by the presence of cytochrome c. All the observed oxidase activities were eliminated by the presence of antimycin A. These results are summarized in Table V.
still be detected. Treatment of the purified complex with chlo-
rate (0.5 mg per mg of protein) rendered the preparation suscepti-
tible to ammonium sulfate fractionation; however, no advantage-
ous separation of the various activities could be observed. In
addition, the TD-transhydrogenase activity obtained by these
procedures in the various fractions was extremely unstable.

Pyridine Nucleotide Analogue Specificity—Table VI summa-
rizes the relative reactivity of TPNH and DPNH with various
oxidized pyridine nucleotide analogue acceptors. The DD-
transhydrogenase is capable of reducing a wide variety of ana-
logues, whereas the TD-transhydrogenase exhibits no reduction
of the 3-propionylpyridine or 3-tritylpyridine analogues of
DPN and low reactivity with the deamino (hypoxanthine) ana-
logue of 3-acetylpyridine.*DPN and corresponding deamino deri-
vant of pyridine-3-aldehyde.*DPN.

Effect of Cd++ and p-Chloromercuribenzoate—As was previously
reported (24), prior incubation of the purified DD-transhydro-
genase with Cd++ or arsenite in the presence of DPNH resulted in
a marked inhibition of this reaction in a manner similar to that
reported by Searls and Sanadi (27) for lipoic dehydrogenase.

TABLE V
Effect of DPN on TPNH oxidase activity of purified beef heart
enzyme complex*  

The 1-ml reaction mixture contained 100 µmoles of potassium
phosphate buffer (pH 7.5), 0.2 µmole of TPNH, and when added,
0.1 µmole of DPN, 0.04 ml of 2% aqueous solution of cytochrome
c, and 1.5 µg of antimycin A in absolute ethanol.

<table>
<thead>
<tr>
<th>Substrate added</th>
<th>Activity µmoles/min/mg protein x 10²</th>
</tr>
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<tbody>
<tr>
<td>TPNH</td>
<td>0</td>
</tr>
<tr>
<td>TPNH + cytochrome c</td>
<td>0</td>
</tr>
<tr>
<td>TPNH + DPN</td>
<td>3</td>
</tr>
<tr>
<td>TPNH + DPN + antimycin A</td>
<td>12</td>
</tr>
<tr>
<td>TPNH + DPN + cytochrome c</td>
<td>0</td>
</tr>
<tr>
<td>TPNH + DPN + cytochrome c + antimycin A</td>
<td>0</td>
</tr>
</tbody>
</table>

* Fraction 5.

TABLE VI
Reactivity of transhydrogenase enzymes with DPN analogues

<table>
<thead>
<tr>
<th>DPN analogue</th>
<th>Relative rates*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DD transhydrogenase</td>
</tr>
<tr>
<td>3-Acetylpyridine†</td>
<td>1.00</td>
</tr>
<tr>
<td>Thionicotinamide</td>
<td>1.30</td>
</tr>
<tr>
<td>Pyridine-3-aldehyde</td>
<td>0.90</td>
</tr>
<tr>
<td>(Deamino) 3-acetylpyridine</td>
<td>0.75</td>
</tr>
<tr>
<td>(Deamino) pyridine-3-aldehyde</td>
<td>0.55</td>
</tr>
<tr>
<td>3-Propionylpyridine</td>
<td>0.50</td>
</tr>
<tr>
<td>3-Butyrylpyridine</td>
<td>0.35</td>
</tr>
</tbody>
</table>

* The rate of the transhydrogenase reaction with 3-acetyl-
pyridine.*DPN was taken as unity. Fraction 5 was used for
enzyme in these studies.
† The following wave lengths (µm) were used to measure the
relative rates of reduction of the pyridine nucleotide analogues:
(1) 375; (2) 400; (3) 365; (4) 375; (5) 369; (6) 365; (7) 365.

Identical results were obtained with the more complex trans-
hydrogenase system. However, the TD-transhydrogenase is rela-
tively insensitive to Cd++ in the presence or absence of its spe-
cific reduced cosubstrate, TPNH. As in the case of the purified
enzymes, the inhibition of the DD enzyme by Cd++ is reversed
only by dithiols. These results are summarized in Table VII.
Similar results were obtained with arsenate.

Table VIII summarizes the effect of prior incubation of the
enzyme complex with p-chloromercuribenzoate. In view of a
possible relationship between the DD-transhydrogenase and
dPNH-cytochrome c reductase (28), the activity of the latter
enzyme was also examined in the presence of this sulfhydryl
inhibitor. The TD-transhydrogenase and DPNH-cytochrome
c reductase are much more sensitive to this reagent than the DD
enzyme. Prior incubation of the enzyme complex with both
p-chloromercuribenzoate and DPNH resulted in a marked in-
crease in the inhibition of the DD activity. In the case of the
TD-transhydrogenase and DPNH-cytochrome c reductase ac-

dition of the appropriate reduced coenzyme to the prior
incubation mixture had no significant effect on the extent of
inhibition. Reduced glutathione completely reversed the
inhibition of the TD activity and to a somewhat lesser extent
reversed the DD activity, whereas this monothiol had no sig-
nificant effect on the inhibition of the DPNH-cytochrome c
reductase reaction. This observation is similar to the findings
of Mahler and Elowe (29) and Lehman and Nason (18). The
TPNH-cytochrome c reductase is further differentiated from the
corresponding DPNH enzyme by the absence of any specific
effect by p-chloromercuribenzoate.

Enzyme was preincubated in cuvette for 10 minutes at room
temperature in 0.1 m potassium phosphate buffer, pH 7.5, con-
taining 10⁻⁴ M CdCl₂. At the end of the prior incubation period
the remaining components of the standard transhydrogenase
assay mixture were added and the reaction was begun by the
addition of the pyridine nucleotide analogue. When added to
the prior incubation medium, reduced pyridine nucleotides were
utilized at the concentrations used in the standard assay mixture.
In measuring the effects of GSH and 2,3-dimercaptopyropro-
panol on inhibition, 10⁻⁴ M concentrations of these compounds were in-
cubated for an additional 5-minute period after the initial 10-
minute prior incubation with cadmium.

<table>
<thead>
<tr>
<th>Prior incubation conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>TD-transhydrogenase</td>
</tr>
<tr>
<td>DD-transhydrogenase</td>
</tr>
<tr>
<td>Cd++</td>
</tr>
<tr>
<td>Cd++ + DPNH</td>
</tr>
<tr>
<td>Cd++ + TPNH</td>
</tr>
<tr>
<td>Cd++ + DPNH + 2,3-dimercaptopropanol</td>
</tr>
<tr>
<td>Cd++ + TPNH + 2,3-dimercaptopropanol</td>
</tr>
<tr>
<td>Cd++ + DPNH + GSH</td>
</tr>
<tr>
<td>Cd++ + TPNH + GSH</td>
</tr>
</tbody>
</table>

* Fraction 5.
It would appear from the nature of the isolation procedure that this preparation consists of small fragments derived from the mitochondrial membran or cristae. With respect to the use of digitonin as a solubilizing agent and the susceptibility of the DP resonance is observed in this system. The decreased sensitivity of the DPN cytochrome e reduc to antimycin A may be explained by a partial modif caty of the enzyme. This is consistent with the relatively low respiratory chain activities observed in this system.

The separation of the DD-transhydrogenase reaction into two fractions only one of which contains the lipo dehydrogenase suggests that the DD enzyme associated with the TD may be specific for the transfer of electrons between free and bound phos pyridine nucleotides of various mitochondrial compartments. The DD-transhydrogenase associated with the lipo dehydrogenase thus corresponds to the enzymes described by Maceev (23) and Searls and Sanadi (30) which were shown by Stei et al. to possess DD-transhydrogenase activity. The second DD enzyme which appears tightly bound in the enzyme complex (Fraction 5) may correspond to the lipo flavoprotein described by Ziegler et al. (31). Attempts to isolate the lipoflavoprotein from Fraction 5 after clutate treatment and solvent extraction according to the method of Ziegler were not successful, although significant amounts of inactive lipid were extracted.

Studies by Weber and Kaplan (28) have shown that purified DPN cytochrome c reductase catalyzes an exchange between DPN and 3-acetylpyridine-DPN which is quite sensitive to inhibition by p-chloromercuribenzoate. The DPN cytochrome c reductase present in this preparation is probably not the source of the exchange reaction, since only moderate inhibition of the exchange reaction is observed when DPN is absent during the prior incubation of the enzyme with the inhibitor. In addition, the marked sensitivity of the TD enzyme to p-chloromercuribenzoate in the absence of TPN during the prior incubation suggests further that the TD and DD reactions are catalyzed by different enzymes. The differences in analogue specificities between the two reactions lead to a similar conclu

**DISCUSSION**

The purified transhydrogenase enzyme preparation obtained from beef heart muscle appears to be a complex of enzymes comprising the major activities concerned with terminal respiration in mitochondria. This intimate structural relationship of the transhydrogenase enzymes with the DPNH electron transport system is further evidence for the importance of DPN in the oxidation of TPNH. Thus, despite the presence of small amounts of TPNH-cytochrome c reductase in this preparation, TPNH oxidase activity could be demonstrated only in the presence of added DPN.

All attempts to separate completely the TD-transhydrogenase enzyme from the cytochrome c reductases and oxidase activities, as well as from the DD-transhydrogenase activity resulted in loss of the TD enzyme. However, the TD reaction is not mediated through the electron transport chain, since it is unaffected by high levels of Amytal and antimycin A. The same conclusion holds true for the DD enzyme. The marked lability of the TD-transhydrogenase to solvents and conditions known to disrupt lipoproteins and phospholipid complexes suggests that the TD activity is in part dependent on the functional integrity of a lipoprotein complex. There does not appear to be an unknown fat-soluble cofactor required for the enzyme, since we have failed to detect such a component in lipid extracts of mitochondria. The enzyme is stable to repeated extractions with isooctane and petroleum ether. This again indicates that a fat-soluble factor may not be involved.

**TABLE VIII**

Effect of p-chloromercuribenzoate on transhydrogenase and cytochrome c reductase activities of purified enzyme complex*

<table>
<thead>
<tr>
<th>Prior incubation conditions</th>
<th>Final concentration</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TD-transhydrogenase</td>
<td>DD-transhydrogenase</td>
</tr>
<tr>
<td>p-Chloromercuribenzoate</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>p-Chloromercuribenzoate (DPNH) 1</td>
<td>0.1</td>
<td>02</td>
</tr>
<tr>
<td>p-Chloromercuribenzoate (DPNH) + GSH</td>
<td>1</td>
<td>78</td>
</tr>
<tr>
<td>p-Chloromercuribenzoate + GSH</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

* Fraction 5.
enzyme is further evidence that the transhydrogenase reaction is the chief route of TPNH oxidation in terminal respiration in animal mitochondria.

**SUMMARY**

1. The reduced triphosphopyridine nucleotide (TPNH)-diphosphopyridine nucleotide (DPN) and the DPNH-DPN transhydrogenase enzymes have been purified approximately 15-fold from digitonin extracts of beef heart mitochondria by means of ethanol precipitation and treatment with calcium phosphate gel. The transhydrogenase activity is closely associated with the mitochondrial respiratory chain, since the purified preparation consists of a complex of the enzymes concerned with terminal electron transport. The purified preparation appears to be devoid of succinic dehydrogenase and pyridine nucleotide-linked dehydrogenases.

2. The activity of the TPNH-DPN (TD) enzyme appears to be dependent on the integrity of a lipoprotein complex, since agents known to disrupt such complexes also inactivate the enzyme.

3. The DPNH-DPN (DD)-transhydrogenase activity has been separated into two fractions, only one of which exhibits lipoyl dehydrogenase activity. The DPNH diaphorase reaction associated with the DD enzyme could be enhanced by mild heating.

4. Both transhydrogenase reactions and the TPNH-cytochrome c reductase were unaffected by large amounts of antimycin A, whereas a marked inhibition of the DPNH cytochrome c reductase reaction was observed. Neither transhydrogenase activity was affected by Amytal; however, both the DPNH- and TPNH-cytochrome c reductases were significantly inhibited.

5. Marked stimulation of both TPNH-cytochrome c reductase and TPNH oxidase activities occurred in the presence of small amounts of DPN. This stimulation was abolished by antimycin A.

6. Inhibition studies with Cd**+** and p-chloromercuibenzoate suggest that the activity of the TD reaction is associated with a sensitive sulphydryl group, whereas the enzymatic activity of the DD reaction appears to be associated with a dithiol group.

7. The relationship of the transhydrogenase reactions described here to terminal respiratory pathways in the mitochondria is discussed.

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**REFERENCES**

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