# PYRIDINE NUCLEOTIDE TRANSHYDROGENASE AND 3α-HYDROXYSTEROID-MEDIATED TRANSHYDROGENASE ACTIVITY OF THE SOLUBLE FRACTION

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The 3α-hydroxysteroid-mediated transhydrogenase (ST) activity of the soluble fraction of rat liver homogenates was reported to be less than 0.05% of pyridine nucleotide transhydrogenase (PNT) activity present in the mitochondria (Stein and Kaplan, 1959). PNT activity in the soluble fraction also exceeded that of ST. It was noted in this study, however, that the ST and PNT activities were dependent upon the conditions of the assay systems. Thus, regardless of its low order of activities, ST still may play a role in vivo.

The enzyme preparation containing ST and PNT were obtained from the soluble fraction of rat liver homogenates, as described by Hurlock and Talalay (1958). The ammonium sulfate fraction precipitated between 50 to 70% saturation was used. The enzyme preparation contained 5.7 mg. of protein per 0.1 ml. The rates of hydrogen transfer from DPNH and TPNH to 3-acetylpyridine analogue of DPN (APDPN) were studied in two assay systems. One consisted of adding free DPNH and TPNH as hydrogen donors and in the other DPNH and TPNH were generated with alcohol and isocitrate dehydrogenases, respectively. APDPN was utilized as the hydrogen acceptor with both systems (Kaplan and Ciotti, 1956). The rates of transhydrogenation were followed directly by the measurement of absorbance at 375 and 400 mu.

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The rate of spontaneous transhydrogenation from DPNH to APDPN due to PNT present in the enzyme preparation was rapid with 0.57 mg. of enzyme protein (Fig. 1). When alcohol dehydrogenase was utilized to generate DPNH,

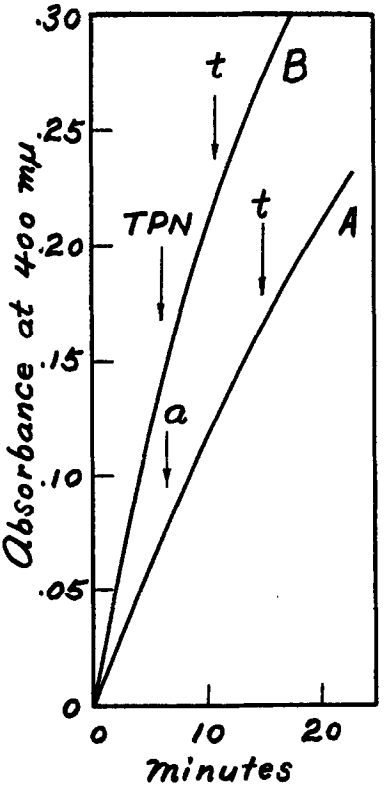


Fig. 1. Effect of androsterone, testosterone and TPN on the rate of hydrogen transfer from DPNH to APDPN. The complete system contained in a volume of 3 ml.: 200 umoles of Tris buffer; pH 7.5; 1.25 umoles of APDPN and 0.5 umoles of DPNH. Systems A and B contained 0.57 and 1.14 mg. protein of 50 to 70% ammonium sulfate fraction of rat liver homogenate, respectively. With system A, 3.4 ug. of androsterone (a) and 6.6 ug. of testosterone (t) in 0.01 ml. of dioxane were added 7 and 15 minutes after the initiation of the reaction. With system B, 0.02 umole of TPN and 6.6 ug. of testosterone (t) in 0.01 ml. of dioxane were added at 6 and 11 minutes after the initiation of the reaction. Measurements were made of absorbance at 400 mμ. against a control cuvette containing all ingredients except APDPN and steroids. Temp. 25°.

the rate of spontaneous transhydrogenation was about 15% of the former system in which DPNH was added. The addition of androsterone or testosterone did not affect the rate in either one of the two systems. Hurlock and Talalay (1958) utilizing a purified enzyme preparation obtained from rat liver homogenates and Baron et al. (1960) using rat and human prostatic extracts showed that hydrogen transfer from DPNH to APDPN was accelerated by the addition of androsterone.

Hydrogen transfer from TPNH to APDPN by PNT occurred rapidly in a 3 ml. system containing 5.7 mg. of enzyme protein (Fig. 2). The addition of androsterone increased the rate of hydrogen transfer only slightly, whereas testosterone had no inhibitory effect. The rate of spontaneous transhydro-

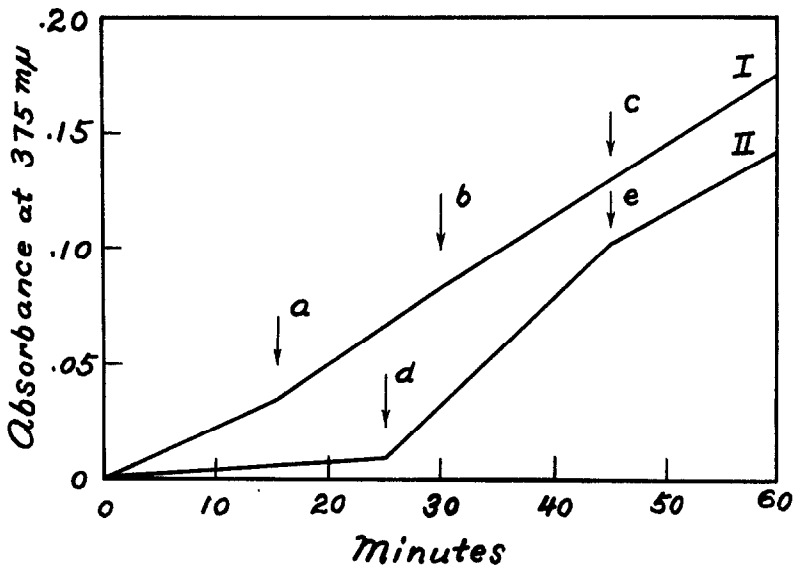


Fig. 2. Effect of androsterone and testosterone on hydrogen transfer from TPNH to APDPN. The complete system I contained in a volume of 3 ml.: 200 umoles of Tris buffer; pH 7.5; 1.25 umoles of APDPN; 0.5 umole of TPNH; and 5.7 mg. protein of 50 to 70% ammonium sulfate fraction of rat liver homogenate. The complete system II contained in a volume of 3 ml.: 200 umoles of Tris buffer; pH 7.5; 1.25 umoles of APDPN; 5 umoles of $MnCl_2$; 10 umoles of isocitrate; 0.02 umole of TPN and 5.7 mg. protein of 50 to 70% ammonium sulfate fraction of rat liver homogenate. 3.4 ug. of androsterone in 0.01 ml. of dioxane were added at points a and d. 6.6 ug. of testosterone in 0.01 ml. of dioxane at points b, c, and e. Measurements were made of absorbance at 375 mu. against a control cuvette containing all ingredients except APDPN and steroids. Temp. 25°.

genation was more pronounced with added TPNH than with TPNH-generated by the addition of isocitrate to the enzyme preparation. The addition of androsterone caused a six-fold increase in the rate of transhydrogenation which was inhibited by testosterone.

These results indicated that with large amounts of TPNH in the system the rate of spontaneous hydrogen transfer by PNT was rapid and ST activity was not demonstrable. In the presence of a TPNH-generating system and a minute amount of TPN, however, hydrogen transfer by ST was significant. Testosterone inhibited hydrogen transfer mediated by ST but had no influence on PNT.

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