A microsomal TPNH-neotetrazolium diaphorase

In the course of previous studies from this laboratory on the site of NT reduction during mitochondrial electron transport[1], one of us (R.H.G.) observed rapid neotetrazolium reduction by microsomes of guinea-pig kidney and rat liver, dependent upon the addition of TPNH or a TPNH-generating system. No activity was observed in the supernatant. Recently, ERNSTER AND NAVAZIO[2] have reported on the distribution in rat-liver cells of diaphorases which reduce DCIP in the presence of DPNH and TPNH. Their greatest activity was in the supernatant fraction; activity in microsomes was considerably higher toward DPNH than toward TPNH.

In view of the marked discrepancy in the apparent "diaphorase" distribution in rat-liver cellular fractions using DCIP or NT, we considered it advisable to assay these activities simultaneously in rat-liver fractions. To make our data comparable to those of ERNSTER AND NAVAZIO, tissue fractionation and DCIP diaphorase assays were performed by their technique and expressed in their units[2]. For NT-diaphorase activity, 0.50 ml of enzyme solution, containing fractions derived from 72 mg of original fresh rat liver, were incubated for 10 min at 37° with 0.4 μmole NT chloride (General Biochemicals, Inc.), 150 μmoles tris(hydroxymethyl)aminomethane, pH 7.45, and 0.63 μmole of DPNH or TPNH in a volume of 1.5 ml. At the end of the incubation, a "pinch" of NaCl and 10 ml acetone were added to each tube, the tubes were shaken and centrifuged, and the absorbance of the supernatant was read in a Coleman Junior Spectrophotometer at 540 mμ. The extinction coefficient of NT and the possibility of production of colored products in intermediate stages of NT reduction have been insufficiently studied; we have used an extinction coefficient obtained by reducing small quantities of dye with an excess of glucose-6-phosphate in the presence of TPN, glucose-6-phosphate dehydrogenase, and microsomes. Under these circumstances the relationship between NT concentration available for "complete" reduction and absorbance of the final acetone solution is linear only at very low concentrations of NT. The results of these assays are indicated in Table I.

TABLE I

INTRACELLULAR DISTRIBUTION OF DPNH AND TPNH DIAPHORASES TOWARD NT AND DCIP

Diaphorase activity (μmoles reduced/min/g original liver).

Fraction	*DCIP*		*NT*	
	DPNH	*TPNH*	*DPNH*	*TPNH*
Mitochondrial	2.6	1.5	0.017	0.023
Microsomal	11.9	1.7	0.015	0.112
Soluble	18.8	23.0	< 0.003	< 0.003

It can be seen from Table I that our findings as to the intracellular distribution of DPNH- and TPNH-DCIP diaphorases are in entire agreement with the data of ERNSTER AND NAVAZIO. In contrast, diaphorase activity toward NT is undetectable in supernatant and maximal in microsomes. It should be noted that NT-diaphorase

Abbreviations: NT, neotetrazolium; DCIP, dichlorophenolindophenol; DPN, DPNH, oxidized and reduced diphosphopyridine nucleotide; TPN, TPNH, oxidized and reduced triphosphopyridine nucleotide.

activity, expressed in μmoles dye reduced/g tissue/min, is much lower than DCIP-diaphorase activity expressed in the same units. Nevertheless, the rate of NT reduction from TPNH in microsomes is fully comparable to the rate of dye reduction from succinate in cytochrome-*c*-fortified liver homogenates.

Our previous studies of the site of NT reduction during electron transport[1] indicated that NT could be reduced at several sites in the electron-transport chain, and that these sites were different when succinate or reduced pyridine nucleotides served as electron donors. A major fraction of NT reduction from succinate in water homogenates of guinea-pig kidney was cyanide and antimycin sensitive and cytochrome-*c* stimulable, suggesting the possibility of NT reduction by iron enzymes. Thus, the possibility may be entertained that NT may serve as a reagent for detecting organized electron transport fragments whose flavoprotein components are inaccessible to dye. Current efforts are being directed toward purification and characterization of this TPNH–NT diaphorase.

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5-Ribosyl uracil, a carbon-carbon ribofuranosyl nucleoside in ribonucleic acids*

The application of chromatographic methods to the examination of ribonucleic acid hydrolysates has resulted recently in the discovery of a variety of "minor constituents" that appear to be bona fide nucleotides[1–4], many of which appear in relatively high concentrations in the so-called "supernatant" fractions.** Although many of these compounds have already been identified as to structure[2], the first to appear (as an unknown peak, labeled ? in Fig. 1 of COHN AND VOLKIN[5]) and to be isolated in quantity and studied (as the "fifth" nucleotide of DAVIS AND ALLEN[1] and nucleotide W of DUNN AND SMITH**) has resisted conventional approaches to structure determination. Recent experiments indicate a unique structure for this substance and also indicate how it (and perchance others of similar structure) may be overlooked or mistaken in some of the usual analytical methods.

The detection, isolation, and many chemical and physical properties of nucleotide W[2] have been described[1,3]. The nucleoside, derived by phosphatase action, does not

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