TABLE II
SPECTROPHOTOMETRIC CONSTANTS OF THE THIONICOTINAMIDE ANALOGS OF DPN AND TPN

	Thionicotinamide-DPN		Thionicotinamide-TPN	
	mμ	A_{mM}[a]	mμ	A_{mM}
		Maxima		
Oxidized[b]	259	19.7	259	19.5
	296[b]	5.0	296[c]	4.9
Reduced[d]	259	16.2	260	15.8
	398	11.9	399	11.7
Cyanide[e]	257	25.1	257	25.6
	359	10.0	359	10.0
		Minima		
Oxidized[b]	236	12.9	236	12.5
Reduced[d]	240	10.8	240	10.7
	324	1.3	324	1.5
A_{400}/A_{340}[f]	5.25		5.34	

[a] Millimolar absorbancy coefficients at the designated wavelengths. [b] Determined in 0.1 M potassium phosphate buffer, pH 7.5. [c] This maximum has been demonstrated by using dense solutions in the region of the maximum (A_{296} = 1.3). [d] Reduced enzymatically; see under Methods. [e] Values determined 20 minutes after the addition of molar potassium cyanide. [f] Absorbancies are for the reduced compounds.

existence of a second maximum at 296 mμ. This peak is altered on reduction.

The spectrophotometric constants of the analogs are shown in Table II. The spectral data for the reduced forms are obtained directly on freshly reduced samples in the dehydrogenase reaction mixtures, the reference cuvet containing all additions save pyridine nucleotide. Complex spectral changes ensue upon addition of the cyanide to the thionicotinamide analogs, consequently the spectra are not presented here. Following a 20-minute interval after addition of the cyanide solution, constant values are obtained and these are presented in Table II. In agreement with the Pabst Laboratories commercial literature,[4] reaction with cyanide markedly increases the ultraviolet absorbancy of the thionicotinamide analogs. This effect appears to be unique among the pyridine nucleotides. The values obtained for the coefficients of the reduced analogs at 400 mμ are about 4–5% higher than those reported by the Pabst Laboratories.[4] We have investigated this problem further by chromatographing a sample of Pabst TNDPN and have estimated the lot analyzed to contain about 4% β-DPN. A comparison of the spectral properties of their product, either published by them or determined by us, with the corresponding sample reported here, leads to the same conclusion.

[4] Circular OR-18, April, 1961.

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The Thionicotinamide Analogs of DPN and TPN. II. Enzyme Studies*

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The reactivity of the thionicotinamide analogs of DPN and TPN in various dehydrogenase and DPNase systems is described. The K_m values of the nicotinamide and thionicotinamide coenzymes obtained in the various dehydrogenase reactions compare very closely, whereas the V_{max} values may show large divergence. In the transglycosidation reaction with pig brain DPNase, the thionicotinamide analogs are converted quantitatively to the corresponding nicotinamide compounds. The thionicotinamide analogs are shown to be reactive with the DPNase from *Neurospora*, either as weak substrates or as very powerful competitive inhibitors of the cleavage of DPN.

The preceding paper in this series (Stein *et al.*, 1963) describes the preparation of the thionicotinamide analog of triphosphopyridine nucleotide (TNTPN)[1] as well as the methods of purification for both this analog and the previously reported (Anderson *et al.*, 1959) thionicotinamide analog of diphosphopyridine nucleotide (TNDPN). The present paper represents a further study of the enzymes employed in the preparation and characterization of the thionicotinamide coenzyme analogs, to provide justification for their use and to provide evidence for the coenzymatic activity of the

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[1] The abbreviations used are: TNDPN, the thionicotinamide analog of DPN; TNTPN, the thionicotinamide analog of TPN; Tris, tris(hydroxymethyl)aminomethane.

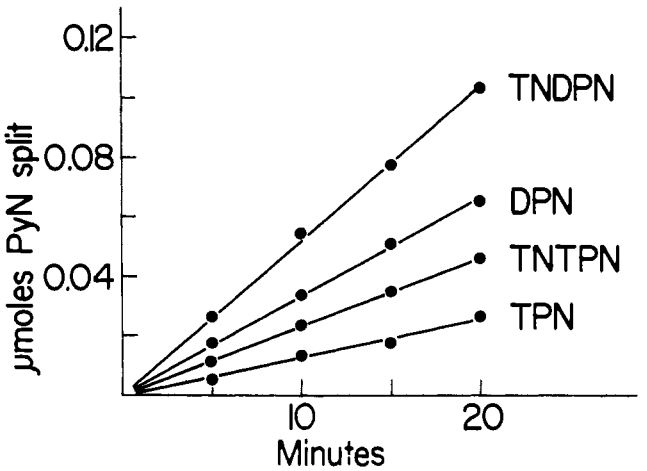

FIG. 1.—Pig brain DPNase-catalyzed hydrolysis of pyridine nucleotides at 25°. Reaction mixtures contained 200 μmoles of potassium phosphate buffer, *p*H 7.5, 8.0 μmoles of the indicated pyridine nucleotide, and 0.4 ml of a suspension of the acid-washed residue of pig brain acetone powder (Stein *et al.*, 1963) in a final volume of 2.0 ml.

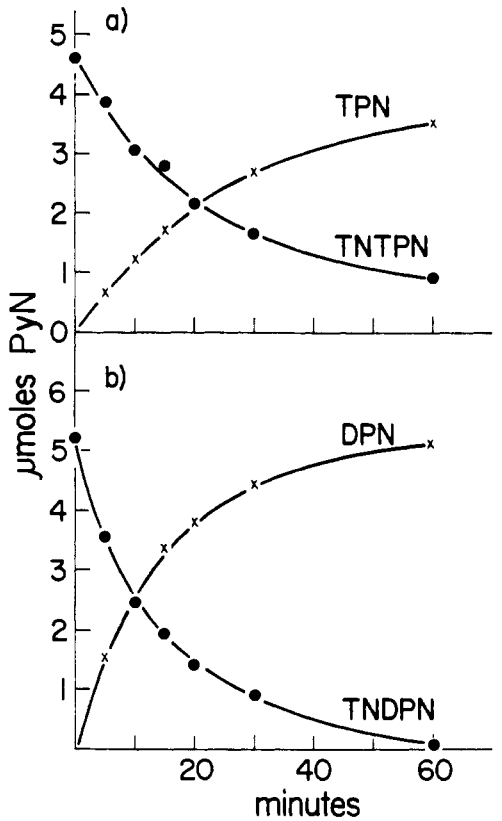

FIG. 2.—Pig brain DPNase exchange reactions at 37°: (a) Reaction mixtures contained 200 μmoles of potassium phosphate buffer, *p*H 7.5, 4.6 μmoles of TNTPN, 100 μmoles of nicotinamide, and 0.4 ml of a suspension of the acid-washed residue of pig brain acetone powder (Stein *et al.*, 1963) in a final volume of 2.0 ml. (b) Reaction mixtures identical to (a) except that 5.6 μmoles of TNDPN were used.

thionicotinamide analog of triphosphopyridine nucleotide. Previous studies have demonstrated thionicotinamide-DPN to function as a coenzyme in several dehydrogenase reactions (Anderson and Kaplan, 1959) and to be successfully applied to the study of the properties of various enzymes (Ciotti *et al.*, 1959; Stein *et al.*, 1959; Kaplan *et al.*, 1960; Dennis and Kaplan, 1960; Kaplan and Ciotti, 1961).

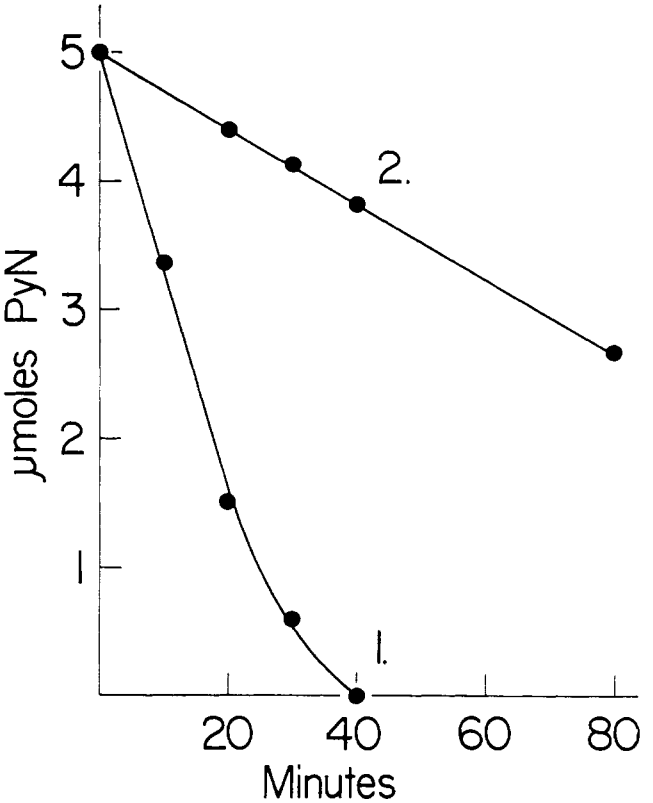

FIG. 3.—The relative effect of *Neurospora* DPNase on DPN and TNDPN: (1) DPN and 130 units of DPNase; (2) TNDPN and 7800 units of DPNase. (A unit defined as amount of enzyme catalyzing the hydrolysis of 0.01 μmole DPN in 7.5 minutes at 37°, Kaplan, 1957.) *Neurospora* DPNase prepared and purified through step III according to Kaplan (1957). Reaction mixtures contained 5 μmoles of pyridine nucleotide. Reactions followed with the yeast alcohol dehydrogenase method at 340 mμ and 400 mμ.

EXPERIMENTAL PROCEDURE

Materials.—Diphosphopyridine nucleotide (DPN) was obtained from Sigma Chemical Company. TNDPN, TNTPN, and pig brain DPNase were prepared as previously described (Stein *et al.*, 1963). The preparation of TPN will be described separately.[2] *Neurospora* DPNase, yeast and horse liver alcohol dehydrogenase (twice crystallized), and rabbit muscle lactic acid dehydrogenase (twice crystallized) were obtained from Worthington Biochemical Corporation. Where indicated, the *Neurospora* DPNase preparation was made by the method of Kaplan (1957). Glucose-6-phosphate dehydrogenase, type II; glucose-6-phosphate, disodium salt; yeast glutathione reductase, type I; and oxidized glutathione were obtained from Sigma Chemical Company. TNTPNH was prepared by enzymatic reduction using the glucose-6-phosphate dehydrogenase system. Pig heart isocitric acid dehydrogenase was prepared as described by Ochoa (1957).

Methods.—The rates of hydrolysis of the various pyridine nucleotides, whether done chemically or catalyzed by pig brain or *Neurospora* DPNase, were measured by methods described by Kaplan (1957). Samples removed from the pig brain DPNase reactions were treated with trichloroacetic acid to precipitate protein and thus avoid turbidity in the analyses for the various pyridine nucleotides. The rates of the dehydrogenase reactions were measured, as usual, by following the formation or disappearance of the reduced pyridine nucleotide spectrum at the appropriate wavelength. Fifteen-second readings were obtained for 4–5 minutes to allow adequate determination of initial

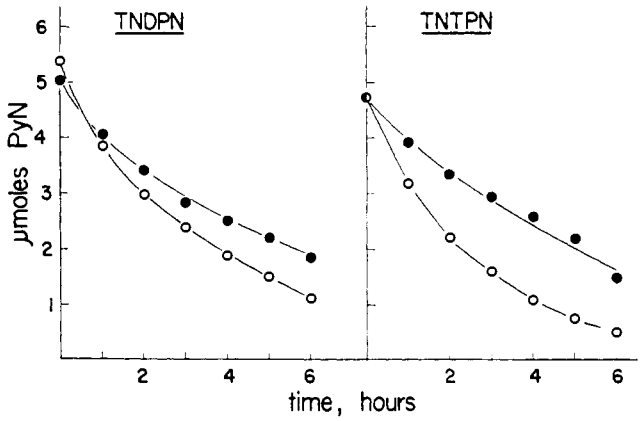

FIG. 4.—The disappearance of pyridine nucleotides in *Neurospora* DPNase reactions as measured by the cyanide addition method (●) and by enzymatic reduction (○). The reaction mixtures contained 5.0×10^{-3} M pyridine nucleotide, 5×10^{-4} M Tris-acetate buffer, *p*H 7.5, and 2600 units *Neurospora* DPNase (a unit defined as amount of enzyme catalyzing the hydrolysis of 0.01 μmole DPN in 7.5 minutes at 37°, Kaplan, 1957) in a total volume of 0.7 ml. Assay by enzymatic reduction was carried out using glucose-6-phosphate dehydrogenase for TNTPN and yeast alcohol dehydrogenase for TNDPN.

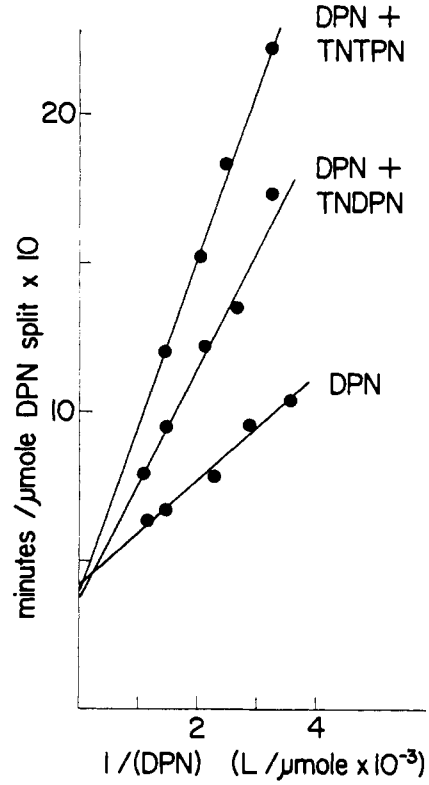

FIG. 5.—Competitive inhibition of *Neurospora* DPNase by TNDPN and TNTPN. Reaction mixtures contained DPN (0.14–0.5 μmole), 50 μmoles potassium phosphate buffer, *p*H 7.5, 0.25 mg *Neurospora* DPNase (Worthington), and either 2.5×10^{-6} M TNDPN or 2.2×10^{-6} M TNTPN as indicated in a total volume of 0.5 ml. Lines are regression curves.

velocities. Yeast alcohol dehydrogenase reactions were studied in 3.0-ml reaction mixtures containing 0.01 M pyrophosphate buffer, *p*H 7.8, 0.1 M ethanol, and pyridine nucleotide concentrations ranging from 2×10^{-5} M to 2×10^{-4} M. Horse liver alcohol dehydrogenase reactions were studied according to Bonnichsen and Brink (1957). Lactic acid dehydrogenase reactions were carried out in 3.0-ml reaction mixtures containing 0.05 M pyrophosphate buffer, *p*H 9.1, 0.075 M sodium lactate, and pyridine nucleotide concentrations ranging from 2×10^{-5} M to 2×10^{-4} M. Isocitric acid dehydrogenase reactions were studied using procedures described by Ochoa (1957). Glucose-6-phosphate dehydrogenase reactions were carried out in 3.0-ml reaction mixtures containing 0.1 M Tris buffer, *p*H 8.5, 4 mg of glucose-6-phosphate, and pyridine nucleotides in concentrations ranging from 1×10^{-5} M to 2×10^{-4} M. Concentrations of the various dehydrogenases were chosen to give initial optical density changes in the region of 0.004–0.025 in 15-second intervals. In the case of yeast alcohol dehydrogenase, approximately twenty times as much enzyme required in the DPN reactions was necessary to maintain these initial velocities when TNDPN was used as the coenzyme. Glutathione reductase reactions were followed by the decrease in absorption of the reduced forms of the pyridine nucleotides studied in reaction mixtures prepared according to Racker (1957).

Rate constants for the chemical hydrolysis of pyridine nucleotides were obtained by plotting the extent of the reaction, $X_\infty - X_t$, against time on semilogarithmic graph paper and by calculating the first-order rate constants from the equation, $k_1 = 0.693/t_{1/2}$.

Spectrophotometric measurements were carried out at 25° on a Zeiss PMQ II spectrophotometer equipped with a thermostatted cell compartment. The isocitric dehydrogenase reactions were carried out in a Gilford Model 2000 recording spectrophotometric system, with the slide wire adjusted to 0–0.1 absorbancy units for full-scale deflection. Measurements of *p*H were made at 25° with a Radiometer *p*H meter, type PHM 4b with a G-200-B glass electrode.

[2] Stein, A. M., Lee, J. K., Anderson, C. D., and Anderson, B. M., unpublished experiments.

RESULTS

Enzymatic Cleavage of Pyridine Nucleotides.—Previous studies (Burton and Kaplan, 1957; Kaplan, 1960; Kaplan and Ciotti, 1956; Anderson, 1958) have shown that some of the pyridine base analogs of DPN prepared by the transglycosidation reaction catalyzed by the pig brain DPNase are, in turn, substrates for the hydrolytic reactions catalyzed by this enzyme. The relative hydrolytic rates of TPN, DPN, and their thionicotinamide analogs are shown in Figure 1. The thionicotinamide analogs prove to be more reactive in this system than their respective parent compounds.

As in the case of the nicotinamide coenzymes, the hydrolysis of the analogs by the pig brain enzyme is inhibited by the constituent pyridine base. In a single experiment, the hydrolysis of thionicotinamide-DPN was inhibited 81% by 0.005 M thionicotinamide. Because of the limited water solubility of thionicotinamide higher concentrations of the base could not be studied. The disappearance of pyridine nucleotides in these reactions has been followed both by measuring the decrease in cyanide reactive material (Stein *et al.*, 1963; Colowick *et al.*, 1951) and by the yeast alcohol dehydrogenase or glucose-6-phosphate dehydrogenase method without any significant differences in rates being observed. TNDPN and TNTPN can also serve as substrates for the transglycosidation reaction catalyzed by pig brain DPNase using nicotinamide as the acceptor for the glycosyl moiety (Fig. 2). These reactions were followed by the reduction of the pyridine nucleotides in yeast alcohol dehydrogenase and glucose-6-phosphate dehydrogenase assays carried out on aliquots from the reaction mixtures after protein precipitation. The presence of 0.05 M nicotinamide accelerated the disappearance of TNDPN and TNTPN over that observed

TABLE I
CHEMICAL HYDROLYSIS OF PYRIDINE NUCLEOTIDES

Pyridine Nucleotide	k_{obs}(min^{-1})
DPN	0.315
TPN	0.262
TNDPN	0.315
TNTPN	0.224

in the hydrolytic reactions; however, at the end of 60 minutes, the conversion of TNDPN to DPN was 98% complete and the conversion of TNTPN to TPN was 76% complete. The data suggest that these exchange reactions occur without hydrolysis under these conditions.

Previous studies with pyridine-base analogs of DPN have indicated (Kaplan *et al.*, 1951) that this class of compounds is not attacked by the powerful DPNase extracted from zinc-deficient *Neurospora* micelium. In contrast to this work with other analogs, we have been able to detect an interaction between the thionicotinamide analogs and *Neurospora* DPNase. Figures 3 and 4 indicate that the thionicotinamide analogs are cleaved by high concentrations of this enzyme at measurable rates, thionicotinamide-DPN being split at about 0.3% the rate of DPN. Figure 4 shows that the major portion of the hydrolytic activity is, indeed, the cleavage of the thionicotinamide ribosidic bond; however, the data provide evidence for another hydrolytic activity in the preparations, presumably a pyrophosphatase. In addition, the thionicotinamide analogs of DPN and TPN are effective competitive inhibitors in this enzyme system (Fig. 5). From the curves in Figure 5 drawn by the method of least squares, a K_m value of 4.3×10^{-4} M was obtained for DPN. This value is in good agreement with the previously reported K_m value of 5×10^{-4} M (Kaplan, 1957). The inhibitor constants (K_I) for TNDPN and TNTPN were calculated to be 6.8×10^{-7} M and 3.9×10^{-7} M, respectively.

The rates of the chemical hydrolyses of DPN, TPN, and their respective thionicotinamide analogs at 100° were obtained in 0.09 M phosphate buffers, *p*H 7.37, with KCl used to maintain the ionic strength constant at 0.4 (Anderson and Anderson, 1963). The observed first-order rate constants for these reactions are listed in Table I.

Dehydrogenase Reactions.—The coenzymatic activity of TNDPN in reactions catalyzed by various dehydrogenases has been demonstrated (Anderson and Kaplan, 1959) and has been employed extensively for the characterization of dehydrogenases (Ciotti *et al.*, 1959; Stein *et al.*, 1959; Kaplan *et al.*, 1960; Dennis and Kaplan, 1961; Kaplan and Ciotti, 1961). The initial reaction rates were plotted according to Lineweaver and Burk (1934) and the K_m values obtained for the analogs and parent coenzymes are shown in Table II along with the maximum velocities relative to the nicotinamide coenzymes. An interesting feature of the data in this table is the similarity between the K_m values of the nicotinamide and thionicotinamide coenzymes for any given dehydrogenase, in contrast to the maximal velocities, which may be greatly different. In the case of yeast alcohol dehydrogenase, the maximal velocity of the reaction with thionicotinamide-DPN is so low, that it might be expected that this analog would prove to be a competitive inhibitor of the dehydrogenase reaction with DPN, as is indeed found to be the case (Table II). The high relative V_{max} of thionicotinamide-DPN in the horse liver alcohol dehydrogenase system contrasts markedly with the low value obtained with the yeast enzyme. Spectral changes observed during the reduction of TNDPN using a wide range of yeast alcohol dehydrogenase concentrations revealed only the gradual development of the reduced form of the analog absorbing maximally at 399 mμ (Stein *et al.*, 1963) and no rapid initial increase in absorption at 330 mμ was detected. These data, with the present, highly purified preparation, fail to confirm the earlier findings.

The functioning of TNTPN as a coenzyme was demonstrated in the reactions catalyzed by glucose-6-phosphate dehydrogenase, glutathione reductase, and isocitric acid dehydrogenase. Difficulties were encountered in the use of TNTPNH as a coenzyme in the glutathione reductase reactions. TNTPNH is sensitive to light and will deteriorate slowly if exposed. A sensitivity to freezing was also observed and, in one instance, a sample of TNTPNH after having been frozen twice could no longer be oxidized in the glutathione reductase reaction. This change occurs without any great effect on the 400 mμ absorption of the compound. Shifrin (1963) has studied the photoreaction of TNDPNH and of 1,4-dihydrothionicotinamide methiodide.

The enzymatic oxidation of TNTPNH by oxidized

TABLE II
THE FUNCTIONING OF THIONICOTINAMIDE ANALOGS AS COENZYMES

Enzyme	Pyridine Nucleotide	K_m (M) Observed	K_m (M) Lit.	Ref.	Relative V_{max}[a]	K_I
Yeast alcohol dehydrogenase	DPN	3.9×10^{-4}	1.7×10^{-4}[b]	(Hayes and Velick, 1954)	1.0	
	TNDPN	4.8×10^{-4}			0.005	
	DPN + TNDPN (2×10^{-4} M)	—	—		—	7.1×10^{-4} (competitive)
Horse liver alcohol dehydrogenase	DPN	8.0×10^{-5}	4.0×10^{-5}[c]	(Theorell *et al.*, 1955)	1.0	
	TNDPN	4.83×10^{-5}			4.9	
Rabbit muscle lactic acid dehydrogenase	DPN	1.9×10^{-4}	2.5×10^{-4}	(Zewe and Fromm, 1962)	1.0	
	TNDPN	2.4×10^{-4}			0.32	
Yeast glucose-6-phosphate dehydrogenase	TPN	2.0×10^{-5}	2.5×10^{-5}	(Glaser and Brown, 1955)	1.0	
	TNTPN	6.4×10^{-6}			0.57	
Glutathione reductase	TPNH	4×10^{-5}[d]			1.0	
	TNTPNH	3×10^{-5}[d]			0.025	
Pig heart isocitric acid dehydrogenase	TPN	3.8×10^{-6}	—		1.0	
	TNTPN	1.5×10^{-5}			0.044	

[a] Maximum velocities obtained by extrapolating initial velocities to infinite pyridine nucleotide concentrations. [b] Determined at *p*H 7.9. [c] Determined at *p*H 10. [d] These values are listed as approximations due to difficulties in obtaining initial velocities over a wide range of pyridine nucleotide concentration.

glutathione is shown in Figure 6. The failure to obtain a greater decrease in absorbancy at 400 mμ and the inability to completely reverse this reaction enzymatically with glucose-6-phosphate are instances of the lability of the compounds reduced under these conditions. It should be emphasized that under the conditions used for the dehydrogenase reaction mixtures, the reduced analogs appear to be quite stable.

Discussion

The reactivity of the thionicotinamide analogs of DPN and TPN in the pig brain DPNase system parallels that of the parent compounds. The analogs undergo hydrolytic cleavage, which is inhibited by thionicotinamide free base, as well as base exchange with free nicotinamide to regenerate the nicotinamide dinucleotides. These data, therefore, permit the formulation of the complete reversible transglycosidation reaction

DPN, TPN + thionicotinamide $\rightleftharpoons$ nicotinamide + thionicotinamide-DPN, TPN

as in the case of the acetylpyridine analog of DPN. Thionicotinamide is, however, a more reactive compound in this system than is acetylpyridine which, at a concentration of 0.01 M, causes only 57% inhibition of pig brain DPNase in the hydrolytic reaction (Anderson, 1958). Similarly, the acetylpyridine-DPN is a much less reactive ribosyl donor to nicotinamide than the corresponding thionicotinamide analog (Anderson, 1958). Although the transglycosidation reaction with the acetylpyridine analog is relatively low, the hydrolytic reaction occurs at a rate comparable to that observed with DPN. TNDPN, on the other hand, is an excellent glycosyl donor to nicotinamide in this transglycosidation reaction yielding stoichiometric amounts of DPN in the presence of 0.05 M nicotinamide (Fig. 2). The pig brain DPNase-catalyzed formation of DPN from nicotinamide and TNDPN proceeds at approximately ten times the rate of DPN formation from nicotinamide and the acetylpyridine analog of DPN under identical conditions (Anderson, 1958). However, only a 2-fold difference was observed for the rate of hydrolysis of TNDPN as compared to that for the acetylpyridine analog (Anderson, 1958). These data are inconsistent with the suggested mechanism for DPNase exchange reactions (Zatman *et al.*, 1954; Zatman *et al.*, 1953) involving a competition between water and pyridine bases for a glycosyl enzyme intermediate after the original pyridine base of the glycosyl donor has been removed. The differences observed in the rates of transglycosidation reactions of various dinucleotides when different acceptors are used indicate that the pyridine moieties of the glycosyl donors exert an effect in the rate-limiting step of these reactions. Such effects would seem unlikely in a reaction involving a competition between acceptors for a common glycosyl enzyme intermediate. Rather, they suggest the displacement of the pyridine moeity of the enzyme-bound dinucleotide by suitable glycosyl acceptors, either another nitrogen base or water. Further studies are necessary to elucidate the role of the pyridine moiety of the glycosyl donor in these transglycosidation reactions.

The low reactivity of *Neurospora* DPNase in the catalysis of the hydrolysis of TNDPN and TNTPN again points to the high specificity of this enzyme (Kaplan, 1957). The very efficient competitive inhibition of DPN hydrolysis by the thionicotinamide analogs indicates a strong interaction between these compounds and the enzyme. Sufficient data are not available to explain the inactivity of the thionicotinamide analogs as substrates for this enzyme; however, the data of Table I indicate that the sulfur-oxygen substitution has little effect on the rates of the chemical hydrolysis of these pyridine nucleotides.

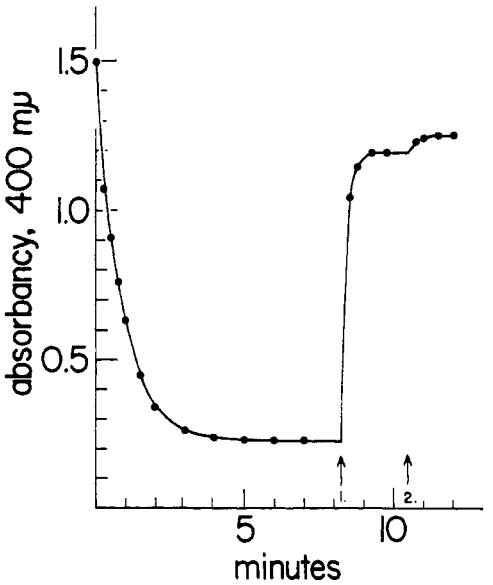

Fig. 6.—Oxidation of thionicotinamide-TPNH by oxidized glutathione and glutathione reductase. Reaction mixtures totaling 1.0 ml contained 50 μmoles potassium phosphate buffer, *p*H 7.6, 0.13 μmole TNTPNH, 0.1% bovine serum albumin, 0.20 mg glutathione reductase, and 0.04 ml of 2% oxidized glutathione which was used to initiate the reaction. At arrow 1, glucose-6-phosphate dehydrogenase and 2 μmoles of glucose-6-phosphate were added. At arrow 2, 1 μmole of glucose-6-phosphate was added.

Because of the high specificity of *Neurospora* DPNase, it has been used successfully in catalyzing the hydrolysis of small amounts of DPN remaining in DPN analog preparations. This procedure was used in previous preparations of TNDPN (Anderson *et al.*, 1959), however more recent observations (Stein *et al.*, 1963) indicate that the unreacted DPN is not removed completely by this *Neurospora* DPNase treatment. The very efficient inhibition by TNDPN and TNTPN of DPN hydrolysis in the *Neurospora* DPNase reactions explains this effect and precludes the use of this enzyme in the purification of the thionicotinamide coenzymes. Any DPN contamination of TNDPN preparations can, however, be removed adequately by fractionation on Dowex-1-formate columns (Stein *et al.*, 1963). Massey and Palmer (1962) have noted a cleavage of thionicotinamide-DPN by *Neurospora* DPNase in a Pabst Chemical Company sample. The rate reported by them is about five times higher than that observed here, and conceivably this might be due to residual DPN (Stein *et al.*, 1963) in the sample.

Previous studies of TNDPN reduction in the yeast alcohol dehydrogenase reaction had revealed a rapid increase in absorption at 330 mμ followed by a gradual development of the TNDPNH peak at 400 mμ (Anderson and Kaplan, 1959). The DPN-free preparations of TNDPN (Stein *et al.*, 1963), when reduced in the yeast alcohol dehydrogenase reaction, reveal only the development of the 400 mμ absorption maximum of TNDPNH.

The utilization of TNTPN as a coenzyme in dehydrogenase systems is demonstrated by the reduction of TNTPN in the isocitric acid and glucose-6-phosphate dehydrogenase reactions; however, the analog appears to be much more efficiently used in the latter enzyme

system. Aside from the difficulties encountered in handling TNTPNH solutions, the reduced analog was oxidized enzymatically in the glutathione reductase reactions. TNTPN, having properties very similar to those of TNDPN, should, as in the case of TNDPN, provide a tool for the study of dehydrogenase reactions.

The striking similarity of the K_m values for the thionicotinamide analogs to those of the nicotinamide coenzymes in the various dehydrogenase reaction mixtures suggests the adenosine diphospho- and adenosine triphosphoribose moieties along with the pyridinium linkages of these compounds are principally involved in coenzyme binding (Fawcett and Kaplan, 1962). Whereas modification of the 3-carbamido function has little or no effect on apparent affinity in the series of dehydrogenases examined here, the coenzymatic activity varies markedly. This is particularly noteworthy in the case of the alcohol dehydrogenases. TNDPN functions at about 0.005 the rate of DPN with the yeast enzyme and at five times the rate in the liver enzyme system.

Conclusions

Highly purified preparations of the thionicotinamide analogs of DPN (TNDPN) and TPN (TNTPN) were studied with respect to the functioning of these dinucleotides as substrates or coenzymes in various enzyme-catalyzed reactions. Both TNDPN and TNTPN serve as substrates for the hydrolytic reaction catalyzed by pig brain DPNase. The order of the rates of the enzyme-catalyzed hydrolyses of DPN, TPN, and the thionicotinamide analogs did not follow the order of rates observed in the chemical hydrolyses of these compounds. Exchange reactions between these dinucleotides and nicotinamide to form DPN and TPN, respectively, as catalyzed by the pig brain enzyme, were demonstrated to proceed stoichiometrically. TNDPN and TNTPN were shown to be very effective competitive inhibitors of the DPN hydrolysis catalyzed by the *Neurospora* enzyme. Hydrolysis of the pyridinium ribosidic bond of the thionicotinamide analogs was shown to be catalyzed by this enzyme, but at a very slow rate as compared to the reaction with DPN.

Further investigations of the functioning of TNDPN in dehydrogenase reactions were presented with the demonstration of competitive inhibition by TNDPN of DPN reduction with yeast alcohol dehydrogenase. On the basis of maximum velocities, TNDPN was reduced at 0.5% the rate of DPN reduction with this enzyme. The functioning of TNTPN as a coenzyme was demonstrated in reactions catalyzed by glucose-6-phosphate dehydrogenase, isocitric acid dehydrogenase, and glutathione reductase. A K_m value of 6.4×10^{-4} M was obtained for TNTPN in the glucose-6-phosphate dehydrogenase reactions and the maximum velocity for TNTPN reduction was shown to be 57% of that observed for TPN reduction with this enzyme. The two other TPN enzymes studied were somewhat less efficient in handling the thionicotinamide analog.

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