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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** Personal communication from Dr. D. B. DUNN.

lend itself to hydrolysis to a base (*e.g.*, perchloric acid, formic acid) or to ribose (prolonged acid hydrolysis, hydrogenation or bromination followed by alkali and acid) although it appears to be a combination of uracil and ribose. This latter conclusion derives from the following evidence: (1) C:N:P = 9:2:1, and there is no amino group; (2) partial release as the free nucleotide by ribonuclease[1]; (3) ionizations detectable by spectrophotometry in the ultraviolet are at pK 9.6 and about 13; the spectral shifts with pH are characteristic of uracil derivatives, particularly of 5-hydroxymethyl uracil; (4) ion-exchange behavior similar to uridine (or uridylic acid); (5) complexing of the nucleoside with borate to the same degree and oxidation at the same rate (and degree) by periodate as uridine; (6) appearance of the nucleoside in snake-venom digests of RNA; (7) addition of two methyl groups by diazomethane as judged by the doubled enhancement of mobility in butanol–water over that of uridine*.

The most conclusive evidence of structure comes from periodate oxidation and nuclear magnetic resonance spectra. The nucleoside reduces 1 mole of periodate[6] to give a product (presumably the dialdehyde, I). This product, treated[6] with excess

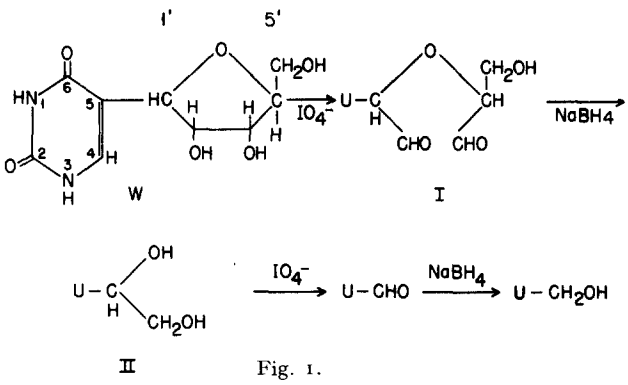

Fig. 1.

$NaBH_4$, gives a partial yield of a substance (II, not identified) that will again reduce 1 mole of periodate to give a product that, reduced with $NaBH_4$, migrates chromatographically with and is spectrophotometrically identical to 5-hydroxymethyl uracil. Neither I nor II is identical to the latter, although II approaches it in chromatographic behavior and spectrum. The degradation is formulated in Fig. 1.

Nuclear magnetic resonance spectra**, run in two different laboratories on a variety of natural and synthetic analogues (uridine, thymine, 4-methyl uracil, 5-hydroxymethyl uracil, 5-hydroxyuridine), indicate in W: (1) a C-4 proton; (2) no C-5 proton; (3) a change in the character of the C-4 proton from that of uridine to that of 5-substituted uracils; (4) a change in the character of the C-1′ proton from that found in uridine (N–C-1′) towards that expected of a carbon-linked CH; (5) a CH_2 group (at C-5′) similar to that of uridine.

The formulation of nucleoside W as "5-ribosyl uracil" helps to explain other properties that could not be explained on the basis of an N-ribosyl linkage, and these explanations serve, in turn, to support the formulation. (1) Strong acid treatment

* These experiments, among others, were carried out by Dr. D. G. DOHERTY.

** Run by LEROY JOHNSON and Dr. JAMES SHOOLERY (Varian Associates) and Dr. D. M. BROWN (Cambridge).

(1 *N* HCl, 1 h, 100°) converts either of the original nucleotide isomers into three or four other isomers that migrate differently on anion exchangers. These may be dephosphorylated enzymically to two additional nucleoside forms that possess degrees of borate complexing markedly differing from the nucleoside derived from the original nucleotides. The differences in borate reactivity among the three nucleoside forms, without loss of carbon, is explained on the basis of furanose, pyranose, and acyclic forms of the ribosyl moiety. (2) The nucleotide (or nucleoside) consumes[7] 1 mole H_2, without loss in u.v. spectrum, but the hydrogenated nucleoside shows negligible complexing with borate, similar to the acyclic form mentioned previously*. The reduced nucleoside is formulated as 5-ribityl uracil. (3) All forms of nucleoside or nucleotide W except the hydrogenated one give, with 1-h heating in the standard orcinol reagent, a red color with a maximum at 540 mμ (not the usual blue green with 660 mμ maximum). This color is also given by the neuraminic acids[8,**]. Only the hydrogenated substance cannot form cyclic anhydrides of the type $HN\text{–}CH{=}CH\text{–}CH{=}CH\text{–}(C)_x$ that neuraminic acids and non-reduced W nucleoside can (and presumably do) form when heated in strong acid. (4) The u.v. spectra of all nucleoside forms approximate those of 5-hydroxymethyl uracil or of thymine and show the two p*K*'s and the two –NH– groups characteristic of those bases.

A C–C ribosyl differs significantly from the usual N–C linkage found in other nucleotides. Conventional modes and concepts of synthesis, of biosynthesis, of analysis, of orientation of active groups in polynucleotide structure[§], and even of nucleotide nomenclature, are not applicable to substances of this type[§§].

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NOTE ADDED IN PROOF

It is proposed (by Dr. A. MICHELSON) that this substance be called pseudouridine, with the symbol Ψ for the prefix "pseudo" in abbreviations. This trivial name seems to serve the needs of exactness and brevity equally well.

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* These experiments, among others, were carried out by Dr. D. G. DOHERTY.
** Dr. Z. DISCHE drew our attention to this phenomenon.
§ However, the 2 and 3 positions can substitute for the 1 and 6 positions in hydrogen bonding.
§§ C-C glycosyls are found in other natural products, however, as in vitexin[9] and barbaloin.[10]
§§§ Operated by the Union Carbide Corporation for the Atomic Energy Commission.