Table 1. Neutral hydrogen distribution in three galaxies.

Galaxy	Neutral hydrogen annulus			Visible edge
	Inside edge	Peak	Outside edge	
Observed radii (kparsec)				
M31	5	9	15	21
Our	4	6.5	12	
M33	2	3.5	5	6
Normalized radii (kparsec)				
M31	5	9	15	21
Our	5.5	9	16.5	
M33	5	9	13	15.5

* Distance to M31 assumed to be 690 kparsec and to M33 630 kparsec.

of the annular distributions in M31, M33, and our galaxy are summarized in the upper part of Table 1. The visual radii for M31 and M33 to 25.8 magnitude per (sec of arc)^2 in blue light as determined by de Vaucouleurs (*9*) are also given. If all three galaxies had the same radius of the hydrogen peak as M31 the radii would be as listed by the normalized values in the lower part of the table. It is apparent from these radii that the neutral hydrogen has a similar relative distribution in the three galaxies. In particular there is a significant deficiency of neutral hydrogen in all three galaxies in the central region. This might indicate a similar evolutionary process in the three galaxies with perhaps an explosion in a prior epoch having swept out most of the neutral hydrogen from the inner regions.

M31, M33, and our galaxy are normal radio galaxies with relatively weak continuum radio emission from the nucleus. By contrast, Mathewson and Rome (*10*) have shown that the nearby galaxies NGC 253 and 4945 have strong continuum emission from the nucleus. It would be of interest to determine whether or not the neutral hydrogen distribution in these galaxies is also deficient in the central regions.

The velocities given in Fig. 2 are with respect to the local standard of rest. The systematic velocity of approach of M31 with respect to this standard is found to be -300 ± 10 km/sec. This is in agreement with Argyle's value of -296 and Roberts' of -310 km/sec.

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7 March 1966

Enzyme-Coenzyme Complexes of Pyridine Nucleotide–Linked Dehydrogenases

Abstract. *Enzyme-reduced coenzyme binary complexes produce previously unreported shifts in the spectrum of the free coenzyme. These shifts give rise to difference spectra which resemble a general environmental change for reduced diphosphopyridine nucleotide (DPNH) in the glutamic dehydrogenase–DPNH complex, and indicate a more specific enzyme-coenzyme interaction for yeast alcohol dehydrogenase–DPNH, isocitrate dehydrogenase–TPNH, and lactic dehydrogenase–DPNH complexes.*

Addition of reduced diphosphopyridine nucleotide (DPNH) to a solution of liver–alcohol dehydrogenase (*1*) or of lactic deyhdrogenase (*2*) results in a shift of the reduced-coenzyme spectrum because of formation of binary enzyme-coenzyme complexes. Since the discovery of these complexes in 1951 and 1952, no such binary complexes have been demonstrated spectrophotometrically for other pyridine nucleotide–linked dehydrogenases, despite vigorous and continued search in many laboratories [although binary and ternary complexes have been shown by fluorometric measurements, and ternary complexes have been observed spectrophotometrically in the presence of substrates or products (*3*)]. Indeed several authors have categorically stated (*2, 4*) that addition of yeast–alcohol dehydrogenase or glutamic dehydrogenase (GDH) to DPNH does not change the DPNH spectrum.

We now show by means of tandem difference – spectrophotometric techniques that formation of spectrophotometrically observable binary complexes between dehydrogenases and DPNH is a more general phenomenon than has been thought; we present evidence of such complexes between reduced coenzyme and GDH, yeast–alcohol dehydrogenase, and isocitric dehydrogenase; and we describe some unusual features of these new signals that may give information on coenzyme-enzyme interactions.

Difference spectra were recorded on a Cary model-14 spectrophotometer using matched 1.000 cm quartz cuvettes arranged as follows: The sample compartment held two cuvettes: the first contained the enzyme; the second, the coenzyme (both in buffer). The reference compartment also held two cuvettes: the first with enzyme and coenzyme mixed, and the second with buffer alone. Artifacts arising from dilution errors, temperature differentials, and stray and scattered light were recognized and avoided by use of the criteria and precautions discussed in (*5*).

Figure 1*A* is the difference spectrum between the GDH-DPNH complex and its components; Fig. 1*B* is a solvent-perturbation difference spectrum of DPNH, presented for reference. This difference spectrum was recorded with the same cell arrangement as for the other difference spectra. In the sample compartment the first cuvette contained DPNH; the second, sucrose (250 mg/-ml). In the reference compartment the first cuvette contained DPNH and sucrose; the second, buffer alone. All cuvettes contained 0.2*M* potassium phosphate buffer, *p*H 7.60. The refractive index difference for the sucrose perturbation of DPNH was 0.0344. The refractive index differences between the enzyme and enzyme-coenzyme solutions were negligible–on the order of 0.0002.

Such difference spectra have been shown to be caused by any change in the immediate environment of the

chromophore; their shape is independent of their immediate cause and is a function only of the spectrum of the chromophore itself (*6*). One can see striking similarity between the effects on the spectrum of DPNH of a relatively small amount of enzyme and of a change in the gross properties of the medium. Figure 1*C* is the difference spectrum between the GDH-TPNH complex and its components; in general it resembles the difference spectrum of the GDH-DPNH complex.

In the GDH-TPNH (reduced triphosphopyridine nucleotide) difference spectrum, the ratio of the absorbance of the nicotinamide signal (>300 mμ) to that of the adenine signal (<300 mμ) is much higher than in the TPNH perturbation-difference spectrum; in the GDH-DPNH difference spectrum this ratio is identical with that of a DPNH perturbation spectrum. This suggests that the nicotinamide moiety of TPNH is bound more strongly to this enzyme than is the adenine moiety, while both moieties of DPNH are bound equally.

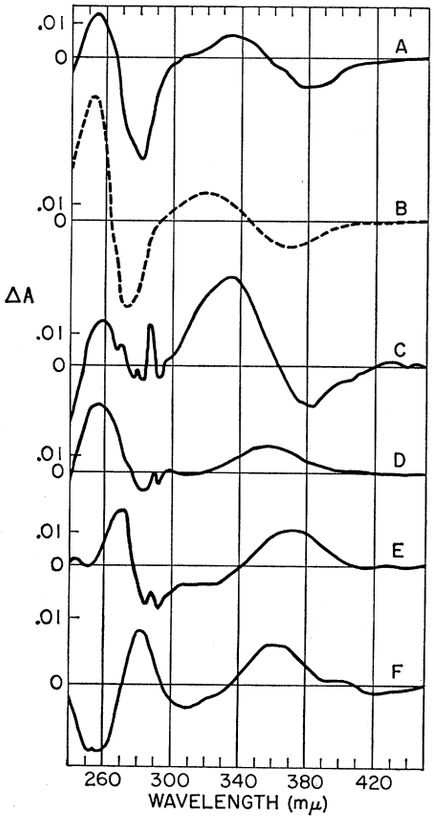

Fig. 1. Absorption-difference spectra of enzyme-reduced coenzyme complexes. *A*, Glutamic dehydrogenase, 1.0 mg/ml, and DPNH, 100 μ*M*; *B*, solvent-perturbation difference-spectrum of DPNH, 200 μ*M*, perturbed by sucrose at 250 mg/ml; *C*, glutamic dehydrogenase, 1.0 mg/ml, and TPNH, 100 μ*M*; *D*, yeast-alcohol dehydrogenase, 1.0 mg/ml, and DPNH, 50 μ*M*; *E*, lactic dehydrogenase, 1.0 mg/ml, and DPNH, 100 μ*M*; *F*, isocitric dehydrogenase, 0.9 mg/ml, and TPNH, 10 μ*M*. With the exception of isocitric dehydrogenase, which was run in tris buffer, all solutions were made up in phosphate buffer at *p*H's near 7.6. Other experimental conditions are described in the text.

The small, sharp, negative peaks at 292 and 284 mμ indicate that in this complex a tryptophan residue is in a region of higher refractive index than in the free enzyme (*5*). Similar tryptophan signals appear in Fig. 1, *D* and *E*, but not in *F*. We have previously reported that the difference spectrum between the inactive ternary complex (GDH-DPNH-glutamate) and the binary complex (GDH-TPNH) shows just such a tryptophan signal, caused in that instance by the binding of the γ-carboxyl group of L-glutamate to a tryptophan residue on the enzyme (*7*); these phenomena will be discussed elsewhere (*8*). Figure 1, *D*–*F*, represents the corresponding difference spectra between enzyme-reduced coenzyme binary complexes and their components for yeast–alcohol dehydrogenase, beef-heart lactic dehydrogenase (*9*), and pig-heart isocitric dehydrogenase, respectively.

The difference spectra of these last three complexes, in contrast with both GDH binary complexes, differ noticeably from the simple perturbation-difference spectrum of DPNH shown in Fig. 1*B*. For example, the difference spectra of both the yeast alcohol dehydrogenase–DPNH and the lactic dehydrogenase–DPNH complexes resemble the perturbation-difference spectrum of DPNH (Fig. 1*B*) throughout the adenine-related portion, but differ markedly from it in the reduced-nicotinamide portion. This fact suggests that in these complexes the interactions between the nicotinamide moiety of DPNH and the enzyme are strong enough and specific enough to affect the chromophore in a manner qualitatively different from a gross medium effect. Yet even these spectra are clearly recognizable as arising from modifications of the adenine and nicotinamide contributions to the spectrum of DPNH.

We do not imply that the complexes demonstrated by these difference spectra necessarily represent active intermediates in the enzyme-catalysed reaction, nor even that they must be located at the active site (*10*); GDH, for example, is known to bind DPNH at noncatalytic sites (*11*). Such assignments must await the correlation of quantitative measurements of each of these signals with the kinetics of their respective enzymatic reactions.

Availability of such spectrophotometric signals, free as they are from many of the ambiguities of spectrofluorometry, may permit more detailed analysis of dehydrogenase mechanisms.

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9. An "approximate" difference spectrum of the lactic dehydrogenase–DPNH complex was obtained by Chance and Neilands (*2*) using single rather than double differential spectroscopy.
10. The 355-mμ peak of the yeast alcohol dehydrogenase–DPNH difference spectrum (Fig. 1*C*) has a measureable time-dependence, although the 255-mμ peak does not. The peak reaches its half-maximum value at about 90 seconds. A slow decrease in absorbance at 340 mμ, when DPNH is incubated with yeast-alcohol dehydrogenase alone, was reported earlier [J. E. Hayes and S. F. Velick, *J. Biol. Chem.* **207**, 225 (1954); K. Wallenfels and B. Müller-Hill, *Biochem. Z.* **339**, 302 (1964)]; presumably these observations are related to the phenomenon that we describe. Thus at least a part of this signal may not represent an obligatory step in the reaction. The GDH-DPNH spectrum (Fig. 1*A*) shows no such time dependence.
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12. Supported by NIH grant GM 11998 and NSF grant GB 3915. We acknowledge the assistance of Lonnie Bevins and Charles Brashear in plotting data, and of Gerald Weatherby in determining the time dependence of the alcohol dehydrogenase–DPNH signal. We thank the Southern Research Support Center of the Veterans Administration for lending a Cary model-14 spectrophotometer.

16 March 1966