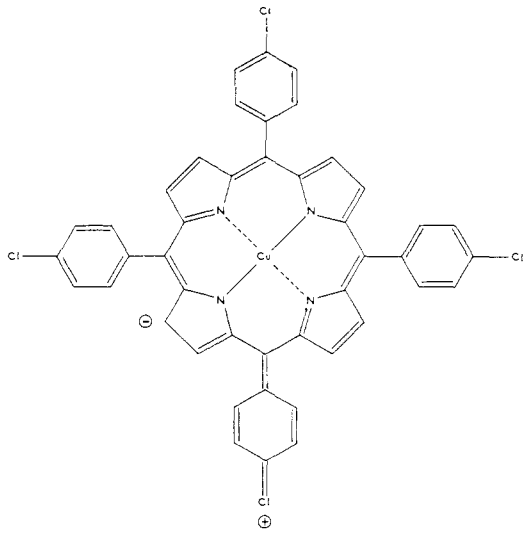

Fig. 2.

phine ring system finds additional support in a recent comparison of the paramagnetic resonance absorption spectra of copper tetraphenylporphyrin and the *p*-chloro derivative. This study suggests that the unpaired electron of the copper atoms can move out to the peripheral Cl- atoms via π orbitals of the conjugated resonating system.[4]

(4) D. J. E. Ingram, J. E. Bennett, P. George and J. M. Goldstein, THIS JOURNAL, **78**, 3545 (1956).

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RECEIVED MAY 9, 1956

AN ENZYMATIC PATHWAY FROM L-XYLULOSE TO D-XYLULOSE[1]

Sir:

The detection of L-xylulose in the urine of "non-pentosuric" humans,[2,3,4] guinea pigs,[3] and rats,[4] together with the discovery of the presence, in guinea pig liver mitochondria, of a highly active enzymatic system for the reduction of L-xylulose to xylitol,[5] indicates that this ketopentose may be a normal metabolite. Recent work[6,7] has shown that D-xylulose-5-phosphate, rather than D-ribulose-5-phosphate, is the ketopentose substrate of transketolase (TK) in the 6-phosphogluconate pathway of carbohydrate metabolism. We wish to describe studies on new mitochondrial enzymes which link metabolically the enantiomorphic forms of xylulose.

A stable, soluble enzyme preparation has been obtained by a butanol treatment of the insoluble portion of ruptured guinea pig liver mitochondria. It contains two enzymes which would make possible the interconversion of L-xylulose and D-xylulose. One enzyme, requiring triphosphopyridine nucleotide (TPN) as coenzyme, uses only L-xylulose and xylitol as substrates. The second enzyme, requiring diphosphopyridine nucleotide (DPN), has D-xylulose and xylitol as its preferred substrates.

The TPN-dependent enzyme catalyzes the dehydrogenation of xylitol but has practically no action on erythritol, ribitol, L-arabitol, D-sorbitol, D-talitol,[8] D-gulitol,[8] or D-mannitol. Oxidation of reduced TPN is effected by L-xylulose, whereas D-xylulose, L-erythrulose,[9] D-ribulose,[10] D-fructose, D-sorbose,[8] and L-sorbose are essentially inactive. Since the L-xylulose-xylitol enzyme has a marked preference for TPN over DPN, it appears to be unique among polyol dehydrogenases (ketose reductases) thus far reported, all of which are DPN-dependent.

The DPN-dependent enzyme catalyzes the oxidation and reduction of xylitol and D-xylulose, respectively. The DPN system acts more slowly on ribitol and D-ribulose but has little or no action on erythritol, L-arabitol, D-talitol, D-gulitol, D-mannitol, L-xylulose, D-fructose, D-sorbose, or L-sorbose.[11]

Figure 1 presents evidence for the existence of two distinct xylitol dehydrogenases. The TPN-dependent enzyme, unlike the DPN-enzyme, loses all or most of its activity by dialysis against water or by preliminary washing of the mitochondrial residue. Table I shows that both dehydrogenases

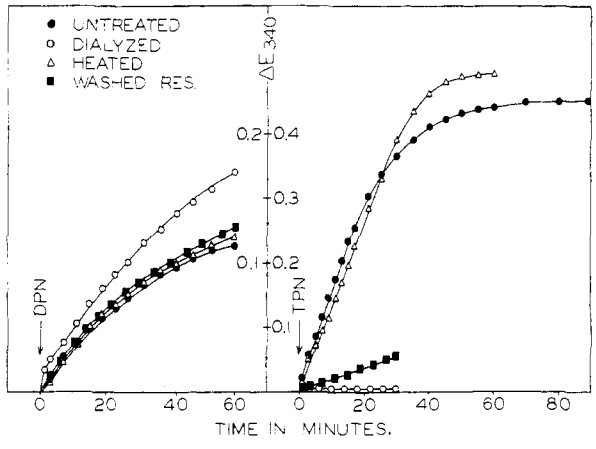

Fig. 1.—Comparison of the two mitochondrial xylitol dehydrogenases: flask contents, 19.7 μM. xylitol, 0.39 μM. coenzyme, 0.3 ml. enzyme, in 3.0 ml. of solution 0.05 *M* to "tris" buffer (*p*H 8.0) and 0.008 *M* to $MgCl_2$; temperature, 23.5°. Dialysis was against water overnight. Heat treatment of enzyme was at 50° for 20 min. "Washed residue" indicates enzyme prepared from insoluble mitochondrial residue which had been washed three times with water before butanol treatment.

(1) Supported in part by a grant from the National Science Foundation.

(2) O. Touster, R. M. Hutcheson and V. H. Reynolds, THIS JOURNAL, **76**, 5005 (1954).

(3) O. Touster, R. M. Hutcheson and L. Rice, *J. Biol. Chem.*, **215**, 677 (1955).

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(5) O. Touster, V. H. Reynolds and R. M. Hutcheson, *J. Biol. Chem.*, in press.

(6) P. A. Srere, J. R. Cooper, V. Klybas and E. Racker, *Arch. Biochem. Biophys.*, **59**, 535 (1955).

(7) B. L. Horecker, J. Hurwitz and P. Z. Smyrniotis, THIS JOURNAL, **78**, 692 (1956).

(8) Kindly supplied by Dr. N. K. Richtmyer.

(9) Kindly supplied by Dr. G. C. Mueller.

(10) Kindly supplied, as the *o*-nitrophenylhydrazone, by Dr. B. L. Horecker.

(11) The extract catalyzes the reduction of DPN in the presence of sorbitol. This reaction is probably due to a third enzyme, since L-sorbose and D-fructose, the expected dehydrogenation products, are inactive. The product may be a 3-ketopolyol.

produce xylulose, in confirmation of conclusions reached from substrate specificity tests.

TABLE I

ANALYSES OF THE XYLITOL DEHYDROGENASE REACTIONS

Reaction[a]	Coenzyme reduced (μM)[b]	Ketose formed (μM)[c]	Red. coenz: ketose	Orcinol test (O.D.)[d] 540/670	435/670
TPN-Xylitol	1.176	1.253	1:1.07	0.38	0.53
DPN-Xylitol	0.947	0.817	1:0.86	.40	.59

[a] Flask contents: 78.8 μM. xylitol, 29.6 μM. coenzyme, and 0.5 ml. of enzyme (1.0 ml. of enzyme extract is derived from the mitochondria of 1.3 g. of liver) in 1.5 ml. of solution 0.05 M to "tris" buffer (pH 9.0) and 0.008 M to $MgCl_2$; reaction time, 180 min. at 36°. [b] Determined by measuring increase in 340 mμ absorption in the Beckman Model DU spectrophotometer. [c] Based on the cysteine–carbazole method[12] (after Ba–Zn deproteinization), with xylulose as standard. The rate of color formation was typical of xylulose. [d] Method of Mejbaum,[13] with 40 min. heating period (Ba–Zn filtrate treated with charcoal to remove DPN). Authentic xylulose: 540/670,0.41; 435/670,0.55; ribulose, 0.75 and 0.66, respectively.

In contrast to particulate preparations, the solubilized enzymes are stable for many weeks at 0°. Both of the xylitol dehydrogenases are completely inhibited by 0.005 M iodoacetate. The substrate requirements as well as the cellular location of the DPN-dependent D-xylulose-enzyme clearly differentiate it from the liver polyol dehydrogenase of Blakley.[14] The enzymes probably provide a bridge between glucuronic acid metabolism and the 6-phosphogluconate pathway, since D-glucuronolactone enhances L-xylulose excretion in mammals.[2,3] The D-xylulose formed from L-xylulose probably would have to be phosphorylated before it could be acted upon by TK, since liver TK[15] has no, and yeast TK[6] only limited, activity on the free ketopentose. The existence of a bacterial kinase which converts D-xylulose to its 5-phosphate derivative[16] opens the possibility for a similar enzyme in mammalian tissues.

(12) Z. Dische and E. Borenfreund, *J. Biol. Chem.*, **192**, 583 (1951).
(13) W. Mejbaum, *Z. physiol. Chem.*, **258**, 117 (1939).
(14) (a) R. L. Blakley, *Biochem. J.*, **49**, 257 (1951); (b) J. McCorkindale and N. L. Edson, *ibid.*, **57**, 518 (1954).
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(17) University of Göttingen School of Medicine, Göttingen, Germany. Fulbright Scholar, 1955–6; aided by a grant from the United States Department of State.

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RECEIVED MAY 10, 1956

THE PARAMAGNETIC RESONANCE SPECTRA OF COPPER PORPHIN DERIVATIVES

Sir:

We recently have investigated the paramagnetic resonance absorption spectra of the copper complexes of $\alpha,\beta,\gamma,\delta$-tetraphenylporphin and its p-chloro derivative, prepared by the methods of Rothemund and Menotti.[1]

Dipole–dipole interaction between neighboring copper atoms in such large molecules is so reduced, even in the concentrated crystal, that the separate hyperfine components of the spectrum can be clearly resolved without resorting to dilution with an isomorphous diamagnetic compound. The hyperfine structure of the unchlorinated derivative consists of four equally-spaced components as in most copper compounds (see Fig. 1A), and the spin Hamiltonian coefficients are

$A = 0.025\,\text{cm.}^{-1}$ $B \approx 0.003\,\text{cm.}^{-1}$ $g_{11} = 2.17$ $g_{\perp} = 2.05$

These values are very close to those obtained for copper phthalocyanine,[2] and A is again considerably greater than that of the octahedral complexes.[3]

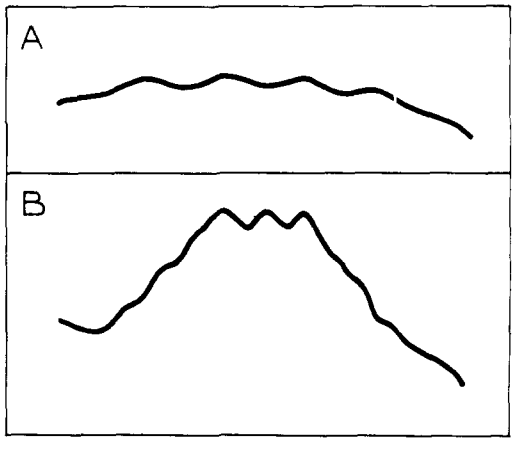

Fig. 1.—Spectra corresponding to the "parallel direction" of A, copper tetraphenylporphin, and B, its p-chloro derivative, from measurements at 20°K. and 36,000 mc./sec. with the same magnetic sweep scale.

However the hyperfine pattern of the chloro derivative no longer consists of four equally-spaced lines but exhibits the standard "christmas tree effect"[4,5] (see Fig. 1B), which shows that there is considerable interaction with the chlorine nuclei causing a further splitting of each hyperfine component. The spectrum has exactly the same general appearance as that first observed with ammonium chloriridate.[4]

Since the splitting is so large, about 100 gauss, it implies that the magnetic electron is associated with the chlorines for an appreciable time. If the chloro derivative crystallizes in such a way that the copper atom of one molecule is close to the chlorine of another, then it is possible that the nuclear interaction is with chlorines of adjacent molecules. But it seems highly unlikely that an electron could be shared between different molecules, because there would be a profound change in g-values from those obtained with copper phthalocyanine if this were so. Hence the results indicate an intramolecular movement of the magnetic electron to peripheral Cl-atoms via the π-orbitals of the conjugated ring system.

The interatomic Cu---Cl distance in this particular molecule can be estimated as about 9–10 Å., and although such a long-range interaction is well known for free radicals[6] where the unpaired

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(2) J. E. Bennett and D. J. E. Ingram, *Nature*, **175**, 130 (1955).
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(6) H. S. Jarret and G. J. Sloan, *J. Chem. Phys.*, **22**, 1783 (1954).