*t*RNA of other species. The most striking upshot is that the three 5-methyluracil methylases have different substrate specificities. They will all operate on *E. coli* *t*RNA, but when a nuclease digest is prepared after methylation, it is found that each enzyme has brought about the methylation of a different uracil. It seems then that the activity of the methylases is not governed merely by factors of steric accessibility, but rather that each enzyme recognizes a particular sequence of nucleotides; these results support earlier work by several groups, indicating that enzymes from one species generally find methylation sites on *t*RNA molecules from other species, which have already been worked over by their endogenous methylases. Svensson *et al.* find again that a yeast uracil-methylating enzyme will work on *t*RNA from the bacterial species *A. aeruginosa*, but not on that from wheat embryo. It is found that all the fractions will insert some methyl groups into ribosomal RNA from *E. coli*, though this, it is suggested, may be a case of breakdown of specificity.

An elevated level of methylase activity has been noted in tumour tissue by Borek and his group and by others. Correspondingly, it was found that whereas there is in general little difference between the *t*RNAs of different tissues, those from tumour cells indeed show markedly changed heterogeneity and chromatographic profiles generally. A further investigation by Baliga *et al.* (*Proc. US Nat. Acad. Sci.*, **62**, 899; 1969) involves the fractionation of *t*RNA from a tumour tissue, Novikoff hepatoma, by chromatography on MAK (methylated albumin-kieselguhr) columns. With healthy liver *t*RNA as the norm, new components were found in the elution profile with histidine, tyrosine and asparagine acceptor activity. Six other species had also evidently undergone some structural change, for they eluted later in the salt gradient than the normal species. It seems likely that differences in methylation are the basis for these observations, but no analyses have yet been performed. It is interesting to note that a high level of methylation of DNA has just been reported in the same tissue (Sneider and Potter, *J. Mol. Biol.*, **42**, 271; 1969): methylation in the 5-position is found to occur in one in every twelve to fourteen cytosine residues. There is evidence of subsequent deamination of the modified base, to give thymine, which, it is suggested, might well have its effect on replication and transcription.

NUCLEAR MAGNETIC RESONANCE

Relaxing Protons

from our Enzymology Correspondent

THE group of workers associated with the names of Albert Mildvan and Mildren Cohn is famous for the ingenuity with which it has pressed nuclear magnetic resonance into the service of enzyme studies. A stream of papers has shown that scrutiny of NMR signal shape and its dependence on time, temperature and magnetic field strength can tell a great deal about the structure and kinetics of enzyme active sites. But some new work by Mildvan and Weiner (*J. Biol. Chem.*, **244**, 2465; 1969) must rank as the most sophisticated excursion of the technique yet seen.

Mildvan and Weiner have managed to make an analogue of the redox coenzyme NAD which bears an unpaired electron in a region corresponding to the pyridine *N*-ribose C_1 bond of the coenzyme. The analogue has a 2,2,6,6-tetramethylpiperidine-1-oxyl group standing in for the usual ribosylnicotinamide, and it has been the key to a fascinating dissection of proton relaxation rates in various complexes of that much-studied but still inscrutable enzyme, liver alcohol dehydrogenase. The unpaired electron in the analogue exerts such a potent effect on the relaxation processes of the protons nearby that a specific NMR study of these protons becomes almost a straightforward matter, though the drawing of firm structural conclusions from the relaxation data remains hazardous.

Three conclusions of Mildvan and Weiner's study are perhaps of special interest. First, ternary complexes of alcohol dehydrogenase with the analogue and substrate are less effective at relaxing solvent water protons than the binary enzyme–analogue complex. This implies that acetaldehyde and ethanol displace water molecules from hydrogen-bonded union with the paramagnetic nitroxide group of the analogue. Second, the analogue alone relaxes the methylene protons of ethanol more than the methyl protons, but in the presence of enzyme the priority is reversed, methyl protons displaying the effect eight to twenty-three times more strongly than those at methylene. The enzyme evidently reorients ethanol with respect to the coenzyme analogue as it forms its ternary complex.

Third, by measuring the temperature dependence of signal broadening, Mildvan and Weiner were able to calculate the distance between the unpaired electron and each relaxing nucleus. Results so far show that substrates bind close to the coenzyme, and on the "water side" of its pyridine ring. The relative position of coenzyme and substrate fits the idea of direct hydrogen transfer from one to the other.

Mildvan and Weiner end their paper with a large ambition. They hope to accumulate a set of spin labelled coenzyme analogues, and measure substrate proton distances from each. By collating the resultant data, they hope to build up a precise picture of the geometry of the catalytically active complex, much as a geographer would use his theodolite to map a peak. Such a map would be of value even for those enzymes that have had the full attention of X-ray crystallography, for it would relate to the act of catalysis in solution.

BIOCHEMICAL DISEASE

Tyrosine and Parkinson's Disease

from our Medical Biochemistry Correspondent

SOME areas of the brain in patients with Parkinson's disease contain much less dopamine than normal brains, and this has promoted interest in tyrosine metabolites. Initial trials of treatment with D,L-dihydroxyphenylalanine (D,L-dopa) were disappointing, but there have been two recent reports that the natural L form does affect the disease. Dopa is formed in the body by hydroxylation of tyrosine, and is readily decarboxylated to dopamine. It was assumed that the deficiency in Parkinsonism was the consequence of low tyrosine hydroxylase activity, and that the administration of dopa would increase the concentration of dopamine and thus restore normal brain function.

Cotzias *et al.* (*New Engl. J. Med.*, **280**, 337; 1969) have recently given gradually increasing doses of L-dopa to twenty-eight patients who were not respond-