one observes better-resolved PMR spectra because of the melting of the secondary structure of the individual tRNA molecules. At all the salt concentrations studied (0.0065*M* to 1.0*M*, *p*D 7 ± 0.2, with or without Mg^{++} at concentrations from 0.01*M* to 0.1*M*), the resolution improvement always occurred over the temperature range 55° to 70°C, with the most significant improvement occurring at approximately 63°C.

Schleich and Goldstein (*5*) have found that the tRNA of *Escherichia coli* is highly aggregated in 1.0*M* NaCl. To demonstrate the presence of aggregates in our preparations, we have run elution profiles on Sephadex G-100 under the same conditions (100 mg of tRNA per milliliter, 1.0*M* or 0.1*M* NaCl, *p*H 6.6) as the PMR experiments. With 1.0*M* NaCl only a strong front-running peak was observed, whereas with 0.1*M* NaCl a weak front-running peak and a strong later-running peak were manifest. The later-running peak coincided with that usually found in 0.1*M* salt for much lower tRNA concentrations. Thus, it appears that, even in 0.1*M* NaCl, there are some aggregates present at the high concentrations of tRNA used for PMR experiments, while at 1.0*M* salt nearly all of the tRNA is aggregated. This accounts for the slight increases in area observed over the temperature range 5° to 20°C for samples with salt concentrations less than 0.75*M* (Fig. 2). Further evidence for aggregation is given by the relative sedimentation rates of unfractionated tRNA (100 mg/ml, *p*H 6.6) in 1.0*M* and 0.1*M* NaCl; in 1.0*M* NaCl the rate is considerably faster than in 0.1*M* NaCl.

We have prepared a sufficient quantity of purified alanine tRNA from yeast to study its PMR behavior. In 0.1*M* NaCl, *p*D 7.0, the areas of regions I and II exhibited temperature dependences similar to those shown in Fig. 2 for 0.02*M* NaCl and 0.0065*M* $MgCl_2$. This demonstrates that the behavior observed for unfractionated yeast tRNA is not due to the presence of impurities introduced by the method of preparation.

In the PMR spectra of unfractionated tRNA at 30°C, it is possible by use of computer averaging to detect very weak broad peaks at 3.07 and 3.90 ppm. Raising the temperature to 67°C results in the appearance of narrow peaks (2 to 5 hz) at 3.94, 3.86, 3.73, 3.25, 3.15, 3.09, 3.03, 2.57, and 2.12 ppm. These correspond to the positions found for the methyl and dihydro groups of the uncommon bases found in tRNA (*6*). With purified alanine tRNA, the high temperature peaks are at 3.80 and 3.10 ppm. The odd bases occur in the loop regions of the proposed cloverleaf model for tRNA (*7*), and thus these high-field resonances provide a valuable structural probe.

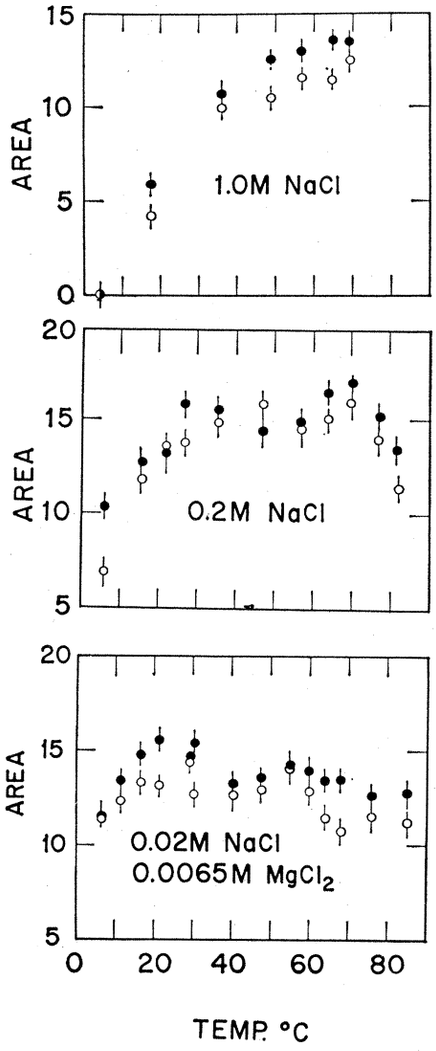

Fig. 2. Variation of the areas under peaks I and II of the 100 Mhz PMR spectra of tRNA with temperature. The filled circles represent peak I (protons in the 1′-position of the ribose moieties and the 5-position of uridine and cytosine); the open circles represent peak II (base protons). The ordinates of the upper plate differ from those in the lower two plates.

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Calcergy Inhibited by Calciphylactic Challengers

Abstract. *The subcutaneous calcification effected in the rat at sites directly treated with calcergens, such as lead acetate, $CeCl_3$, $CaCl_2$, and $KMnO_4$, is inhibited by simultaneous local application of various calciphylactic challengers, but not by many other compounds.*

Calciphylactic challengers produce calcification of soft tissues at the sites of their administration only after sensitization by systemic treatment with such calcium-mobilizing factors as parathyroid extract, vitamin D, or dihydrotachysterol; calcergens exert the same effect without any previous sensitization. Thus calciphylaxis is, while calcergy is not, dependent upon sensitizing alterations in systemic calcium and phosphate metabolism. However, the end results of both reactions are the same: deposition of hydroxylapatite which forms a readily visible white disk at the site of injection of the provocative substance (*1*).

Recently we observed that topical calcification is prevented in rats if calcergens are injected simultaneously with calciphylactic challengers, whereas agents that do not cause calcification upon subcutaneous injection, either in unpretreated or in calciphylactically sensitized rats, fail to protect against the topical calcium deposition normally elicited by calcergens. This apparently paradoxical fact further emphasizes the essential dissimilarity of calciphylaxis and calcergy.

Female Sprague-Dawley rats averaging 100 g (90 to 110 g) were divided into groups each of 5 to 10 animals and injected subcutaneously under the

shaved skin of the back with 200 μg of lead acetate, cerium chloride, or potassium permanganate, or 2 mg of calcium chloride, in 0.5 ml of distilled water. Preliminary experiments had shown that at these dosages all the agents tested produce calcified disks having a mean diameter of about 10 mm.

Each rat received one of these calcergens at one point alone and, at a distance of 2 to 3 cm, conjointly with a test substance. In the cases of lead acetate, cerium chloride, and calcium chloride, the calcergen and the test substance were mixed in 0.5 ml of water, whereas potassium permanganate (which caused precipitates with many of the test substances) had to be injected 3 minutes before the potential inhibitor (each in 0.25 ml of water) at the same spot.

The animals were kept on Purina Fox Chow during the experiment and killed with chloroform on the 5th day. The diameters of the resultant disks were averaged, and the calcific nature of the deposits was subsequently verified histochemically (von Kóssa and chloranilic acid tests).

The histologic details and the statistical evaluation of the data will be published elsewhere, but, since in most instances the calcification was either maximal or nil, the results can be easily summarized as follows:

At doses of 500 μg there was no inhibition by AgCl, $CeCl_3$, CsCl, $CuCl_2$, $HgCl_2$, KCl, KI, LiCl, $MgCl_2$, $MnCl_2$, NaCl, Na_2PtCl_6, NH_4Cl, $NiCl_2$, RbCl, Re_2O_7, SeO_2, $SrCl_2$, or $TlCl_3$, each of which had been shown not to act as a calciphylactic challenger in appropriately sensitized rats (*1*). On the other hand, the following verified calciphylactic challengers completely, or virtually completely, inhibited the calcification normally induced by any of our four calcergens: $AlCl_3$, $CrCl_3$, $FeCl_2$, $FeCl_3$, $SnCl_2$ (each at a dose of 500 μg), aluminium dextran (equivalent to 9.7 mg of aluminium), chromium dextran (9.85 mg of chromium), ferric dextran (6.25 mg of iron), ferric dextrin (2.5 mg of iron), ferric oxysaccharate (2.5 mg of iron), and ferric sorbitol (2.5 mg of iron).

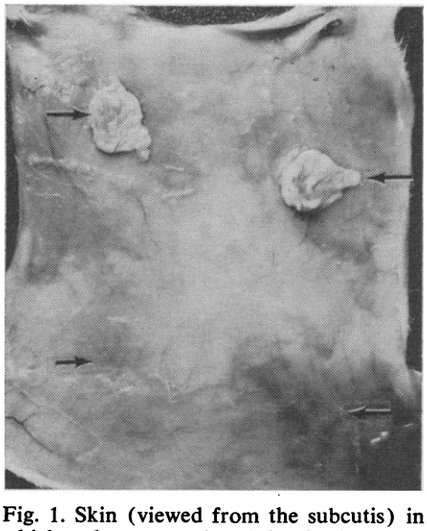

Fig. 1. Skin (viewed from the subcutis) in which calcergy produced by lead acetate (top left) and potassium permanganate (top right) is inhibited by ferric dextran given conjointly with the same agents (bottom left and bottom right, respectively).

Figure 1 shows macroscopically the skin of a rat in which the calcifications that are normally produced by lead acetate and $KMnO_4$ were inhibited by ferric dextran.

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Interconversions of Mitochondrial Pyridine Nucleotides

Abstract. *Among the factors that can govern the relative rates of mitochondrial oxidation of isocitrate by means of the isocitrate dehydrogenases linked to diphospho- and triphosphopyridine nucleotides are the intramitochondrial concentrations of these nucleotides. Yeast mitochondria contain enzymes that can alter the ratio of these pyridine nucleotides by interconverting them. A diphosphopyridine nucleotide kinase catalyzes the formation of triphosphopyridine nucleotide from diphosphopyridine nucleotide and adenosine triphosphate; a phosphatase converts triphosphopyridine nucleotide to diphosphopyridine nucleotide. Both reactions are more active with the reduced forms of the pyridine nucleotide coenzymes. These studies suggest that the activity of the mitochondrial triphosphopyridine-nucleotide–linked isocitrate dehydrogenase may be regulated by a cyclic system of pyridine-nucleotide interconversions involving triphosphopyridine nucleotide synthesis and breakdown.*

The oxidation of isocitrate to α-ketoglutarate and CO_2 in mitochondria can proceed by either TPN-linked or DPN-linked isocitrate dehydrogenase (TPN, triphosphopyridine nucleotide; DPN, diphosphopyridine nucleotide). These two pathways have been demonstrated with mitochondria from tissues of rat, pigeon, and locust (*1*, *2*), and from guinea pig heart (*3*) and yeast (*4*). Although the regulation of the DPN-linked pathway of isocitrate oxidation has been described (*5*, *6*), little is known of the mechanism which controls the TPN-linked pathway. We have suggested that the intramitochondrial concentration of TPN might be responsible for regulating the TPN-linked pathway (*4*). This was based on kinetic data which indicated that significant amounts of TPN are either not present in isolated yeast mitochondria or not accessible to the TPN-linked isocitrate dehydrogenase.

Intact yeast mitochondria were prepared as previously described (*4*). Pyridine nucleotides were extracted with perchloric acid (*7*) after oxidation with 5-ethylphenazinium ethyl sulfate and air (*8*). This procedure yields the total content of the pyridine nucleotides without regard to their original state of reduction. The coenzymes were then assayed enzymatically: DPN with ethanol and alcohol dehydrogenase from yeast, and TPN with isocitrate and TPN-specific isocitrate dehydrogenase from pig heart (*9*). Protein was measured according to the method of Lowry *et al.* (*10*).

All incubations were conducted in test tubes (18 by 65 mm) immersed in a 30°C water bath, and the reactions were initiated by introduction of mitochondria. Mixing was done with small magnetic stirring bars propelled by a submerged motor; and the vessels were exposed to air, except when reduced pyridine nucleotides were present. In the latter cases, the reaction mixtures were kept anaerobic with a stream of water-saturated nitrogen to prevent oxidation of the reduced pyridine nucleotides by the mitochondrial preparations.

Direct analysis of mitochondrial suspensions gave the following values for the pyridine nucleotides: DPN + DPNH = 2.71 ± 0.61; TPN + TPNH = 0.43 ± 0.18 nmole per milligram of