groups and other structural defects all support the *cis*-syndiotactic double chain structure, and indicate that 95–98% of the phenyl-T units in the higher polymers are in the *cis*-syndiotactic configuration. This arrangement of trifunctional units into a linear, double chain network is reminiscent of that found in DNA. Calculations of bond angles and examination of models show that the *cis*-syndiotactic ladder structure is the only extended, regular network of three-connected units which can accommodate the rigid, bulky phenylsilsesquioxane groups while maintaining tetrahedral bond angles on the silicons and the preferred bond angles of *ca.* 155°[4] on the oxygens.

The results show that the formation of an ordered one-dimensional network (double chain polymer) under equilibrium controlled conditions can be a spontaneously stereoselective process, probably for much the same reasons that the formation of ordered three-dimensional networks (crystals) can also be spontaneously stereoselective. In both cases the requirement that each structural building unit be at a bridgehead position, joined to three or more others in such a way as to avoid bond angle distortions or steric hindrance, places severe limitations upon the permissible modes of bond formation. It is suggested that this inherent stereoselectivity of network structure formation may help account for the evolution and operation of the network polymer systems which play such prominent roles in living organisms.

(4) R. F. Curl and K. S. Pitzer, THIS JOURNAL, **80**, 2371 (1958).

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THE POSSIBLE PARTICIPATION OF FLAVIN ADENINE DINUCLEOTIDE IN ADENOSINETRIPHOSPHATASE ACTIVITY[1]

Sir:

Recent reports of the effects of reducing agents[2] and flavin antagonists[3] on the ATPase[4] activity of mitochondria, and subparticles therefrom, indicate that a flavin derivative is possibly involved in the process. Because of the importance attached to ATPase as a manifestation of the system of oxidative phosphorylation, it was decided worthwhile to investigate the question of flavin involvement with the soluble ATPase of desiccated mitochondria.[5]

Materials and Methods.—Livers of adult, male Sprague–Dawley rats were the source of mitochondria isolated by conventional procedures. The mitochondria were converted to acetone powders immediately after isolation. Extracts of the powders were obtained by homogenizing in 0.25 *M* sucrose, and retaining that portion of the solution unsedimented by centrifugation at 105,000 *g* for 60 min. For maximal utilization, the extracts were lyophilized and reconstituted in water as needed. Enzyme activity was determined by the Martin and Doty procedure.[6] The FAD was of 99% purity.[7]

Results.—Previous work had shown that the ATPase of these preparations was greatly stimulated by dithionite and agents such as $NaHSO_3$ and NaHS. This effect indicated a possible requirement for a reducible cofactor or functional group. Oxidized and reduced DPN, TPN and cytochrome c had no effect on ATPase activity. However, experiments with FMN and FAD yielded rather dramatic results.

Figure 1 shows the effects of FAD on the ATPase activity in the presence of Mg^{++} or DNP. Pi formation with either activator is definitely elevated in the presence of added FAD over a range of FAD concentrations. In both cases the stimulation is maximal at 4.4×10^{-4} *M* FAD, and can be detected at the lowest concentration used (3.6×10^{-5} *M*). In contrast, FMN at these and higher concentrations has no discernible effect. Control experiments indicate that FAD causes no Pi formation in the absence of ATP.

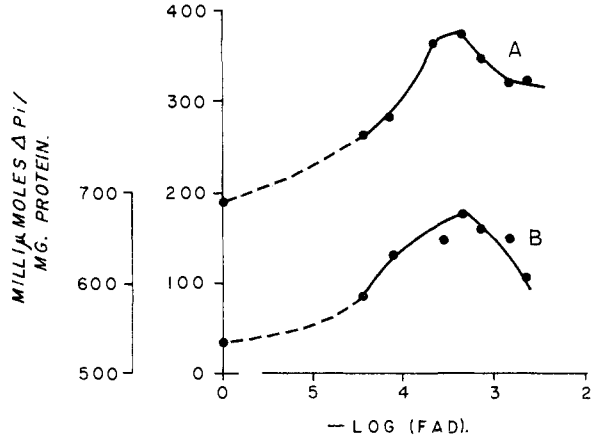

Fig. 1.—Stimulation of ATPase by added FAD—conditions: 6×10^{-2} *M* Tris buffer; 0.75 mg. protein; 1.2×10^{-2} *M* ATP; in volume 0.5 ml.; incubation temp. is 27°; curve A, 1×10^{-3} *M* DNP, *p*H 8.5, 10 min. incubation; curve B (accessory ordinate), 6×10^{-3} *M* Mg^{++}, *p*H 9.0, 4 min. incubation.

Table I shows that this phenomenon is reproducible with this type of preparation. In each of the four extracts tested, there is a definite stimulation by FAD which may be as great as 100%. One problem encountered with the soluble ATPase is loss of the DNP and Mg^{++} responses, particularly the former, under a variety of storage conditions. It was hoped that this process could possibly be due to dissociation or destruction of FAD.

(1) Supported in part by a National Science Foundation Grant (G-7490); North Carolina Heart Association; Institutional Grant of American Cancer Society; UNC University Research Council.

(2) (a) D. K. Myers and E. C. Slater, *Biochem. J.*, **67**, 558 (1957); (b) H. Löw, *Biochim. Biophys. Acta*, **32**, 1 (1959).

(3) (a) H. Löw, *ibid.*, **32**, 11 (1959); (b) M. J. R. Dawkins, J. D. Judah and K. R. Rees, *Biochem. J.*, **76**, 200 (1960).

(4) Abbreviations: ATPase, adenosinetriphosphatase; DNP, 2,4-dinitrophenol; DPN and TPN, di- and triphosphopyridinenucleotide; FMN, flavin mononucleotide; FAD, flavin adenine dinucleotide; Pi, inorganic phosphate; ATP, adenosinetriphosphate.

(5) H. A. Lardy and H. Wellman, *J. Biol. Chem.*, **201**, 357 (1953).

(6) L. Ernster, in "Methods of Biochemical Analysis," Interscience Publishers, Inc., New York, N. Y., **3**, 1 (1956).

(7) Obtained from California Corp. for Biochemical Research.

TABLE I

EFFECTS OF FAD ON SOLUBLE DNP AND Mg^{++} ATPASE ACTIVITY

Conditions: 23 μmoles Tris, pH 8.5 and 9.0 in presence DNP and Mg^{++}, respectively; 6 μmoles ATP; 0.3–0.75 mg. of protein, in volume 0.50 ml. Incubated 10 min. and 4 min. in presence of DNP and Mg^{++}, respectively, at 28°.

	Millimoles Pi liberated/mg. protein					
	1×10^{-3} DNP			6×10^{-3} M Mg^{++}		
Extract FAD:	...	0.00045 M	0.0045 M	...	0.00045 M	0.0045 M
L-6	193	221	218	495	605	646
L-7				456	475	514
L-11				421	520	508
L-10	190	375		535	676	
L-10[a]	138	248		423	476	

[a] Preparation aged 48 hours at −17°.

With an aged extract FAD is still stimulatory, but it is incapable of restoring the original level of activity. However, part of the activity loss may be attributed to the existence of a multiplicity of ATPase entities in these extracts,[8] only one of which involves a system employing a flavin moiety, but all of which involve Mg^{++}.

As with mitochondria and particulate preparations, the soluble ATPase is strongly inhibited by flavin antagonists such as atebrin and chloropromazine. However, the effects of such materials on the ATPase of these preparations differ in several respects from those observed with the particulate preparations[9]: (1) ATPase activity is inhibited in the presence of DNP or Mg^{++}, there being no evidence of a stimulatory concentration range in the presence of DNP.[9a] (2) Amytal does not reverse the effect of atebrin, but rather augments it.

These results provide the first direct evidence for the possible involvement of a flavin (FAD) in ATPase activity. They differ from results obtained on a soluble Mg^{++}-ATPase derived from sonicated mitochondria.[9] It must be presumed that desiccation yields a more complex molecule or system which employs FAD as a possible cofactor or prosthetic group. Work is being continued to further detail the function of FAD in ATPase activity.

(8) R. Penniall, *Biochem. Biophys. Acta*, in press.
(9) R. E. Beyer, *ibid.*, **41**, 552 (1960).
(10) Fellow of the American Heart Association.

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TRANSITION POINT DEPRESSION OF CESIUM CHLORIDE BY RUBIDIUM CHLORIDE

Sir:

A previous note[1] indicated that the observed transition point depression of cesium chloride by rubidium chloride[2] cannot be reconciled with the recently observed heat of transition, ΔH_{tr}, of cesium chloride.[3] This warrants a closer examination of the relation between these quantities, given by the equation

$$\Delta\bar{H} - T_m\Delta\bar{S} = -\frac{\Delta H_{tr}}{T_{tr}}(T_{tr} - T_m) + T_m\int_{T_{tr}}^{T_m}\frac{dT}{T^2}\int_{T_{tr}}^{T}\Delta c_p dT \quad (1)$$

Here $\Delta\bar{H}$ and $\Delta\bar{S}$ are the relative partial molar heat and entropy, respectively, of cesium chloride in the solid solution saturated with cesium chloride at the temperature T_m. T_{tr} is the transition temperature of pure cesium chloride and Δc_p is the difference in specific heat of high and low cesium chloride. The above expression simply amounts to equating the free energy of pure low cesium chloride at the temperature T_m with the partial free energy of cesium chloride in the saturated solid solution at T_m. Pure high cesium chloride supercooled to T_m is taken as the standard reference state. The equation only requires that the observed condition of no solid solubility of rubidium chloride in low cesium chloride[2] prevails up to T_{tr}. (For further details, the completely analogous treatment of a liquidus curve might be consulted.[4])

In the earlier paper,[1] these approximations were introduced in equation 1

$$\Delta\bar{H} = 0 \quad (2)$$

$$\Delta\bar{S} = -\mathbf{R}\ln p \quad (3)$$

$$\Delta c_p = 0 \quad (4)$$

Here p is the mole fraction of cesium chloride in the solid solution and $\mathbf{R}$ is the gas constant. It is not obvious, however, that these approximations are permissible. In view of the apparent discrepancy of the transition point depression curve with the heat of transition it would be of interest to obtain numerical values for $\Delta\bar{H}$, $\Delta\bar{S}$ and Δc_p. A theory by Wasastjerna[5] (with modifications by Hovi[6]) for the integral heat of mixing of solid alkali halides may be used to derive fairly reliable expressions for $\Delta\bar{H}$ and $\Delta\bar{S}$. Starting with Hovi's equations[6] and the definition of partial molar quantities, these equations have been deduced

$$\Delta\bar{H} = \frac{NCe^2\Delta R}{R^3}\left[(1-p)^2\left\{1+(1-2p)\frac{3\Delta R}{R}\right\}\left\{\frac{\vartheta}{4}(1-\sigma)+\frac{1}{2}(1+\sigma)\right\} + \frac{(1-p)(1-2p)\sigma}{1-2(1-e^n)(1+\sigma)p(1-p)}\left\{1+(1-p)\frac{3\Delta R}{R}\right\}\left\{\frac{1}{2}-\frac{\vartheta}{4}\right\}\right] \quad (5)$$

$$\Delta\bar{S} = -\mathbf{R}\left[\ln\{p-(1-p)\sigma\} - p(1-p)(1+\sigma)n - \frac{(1-2p)(1-p)n}{1-2(1-e^n)(1+\sigma)p(1-p)}\right] \quad (6)$$

In these equations N is the Avogadro number. C is the Madelung constant, e the charge of an electron, R the equilibrium distance between cesium and chlorine nearest neighbors in the lattice and ΔR the difference between R and a corresponding quantity for the rubidium chloride lattice. The three quantities σ, n and ϑ are given by the equations[6]

(1) J. Krogh-Moe, *J. Am. Chem. Soc.*, **82**, 2399 (1960).
(2) L. J. Wood, C. Sweeney and M. T. Derbes, *ibid.*, **81**, 6148 (1959).
(3) C. E. Kaylor, G. E. Walden and D. F. Smith, *J. Phys. Chem.*, **64**, 276 (1960).
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(6) V. Hovi, *ibid.*, **XV**, No. 12 (1950).