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°.

	Milliμmoles 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[3]: (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.[3a] (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.

DEPARTMENT OF BIOCHEMISTRY
SCHOOL OF MEDICINE
UNIVERSITY OF NORTH CAROLINA
CHAPEL HILL, N. C.
RALPH PENNIALL[10]

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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 **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]

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[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).

[4] C. Wagner, "Thermodynamics of Alloys," Addison-Wesley Press, Cambridge, Mass., 1952.

[5] J. A. Wasastjerna, *Soc. Sci. Fenn. Comm. Phys.-Math.*, **XV**, No. 3 (1949).

[6] V. Hovi, *ibid.*, **XV**, No. 12 (1950).

$$\vartheta = \frac{3 + 4\dfrac{T}{V}\left(\dfrac{\partial V}{\partial T}\right)_p}{\dfrac{\mathrm{C}e^2}{6R^4V}\left(\dfrac{\partial V}{\partial p}\right)_T - \dfrac{T}{V}\left(\dfrac{\partial V}{\partial T}\right)_p} \qquad (7)$$

$$n = \frac{NCe^2\Delta R^2}{\mathbf{R}TR^3}\left(\frac{1}{2} - \frac{\vartheta}{4}\right)\left(1 + (1-p)\frac{3\Delta R}{R}\right) \qquad (8)$$

$$\sigma = \frac{1 - \sqrt{1 - 4p(1-p)(1-e^n)}}{2(1-e^n)p(1-p)} - 1 \qquad (9)$$

ϑ can be calculated if the compressibility coefficient, $\frac{1}{V}\left(\frac{\partial V}{\partial p}\right)_T$, and the cubic expansion coefficient, $\frac{1}{V}\left(\frac{\partial V}{\partial T}\right)_p$, is known. Equations 5 and 6 have been simplified by assuming that the ϑ value for cesium chloride is equal to the value for rubidium chloride. This is not likely to introduce a serious error, since the values calculated for some other alkali halides indicate little variation in ϑ.[7]

Numerical values for $\Delta\bar{H}$ and $T\Delta\bar{S}$ have been evaluated from equations 5–9, taking for ϑ the value 9.1 given by Hovi for rubidium chloride,[7] and calculating R and ΔR at various temperatures for the unit cell edge interpolation formula given by Wood, Sweeney and Derbes.[2] Results are given in cal./mole in the table, columns 3 and 4, for a solid solution of p mole of cesium chloride with $1-p$ mole % rubidium chloride at the temperature of initial transition. Column 5 gives the simpler expression $\mathbf{R}T \ln p$.

TABLE I

T_m, °K.	p	$\Delta\bar{H}$	$T\Delta\bar{S}$	$\mathbf{R}T \ln p$
735	1.00	0	0	0
711	0.95	4	73	73
668	0.90	14	141	139
628	0.80	54	282	278
488	0.50	251	652	672

These data show clearly that the approximations represented by equation 2 and 3 did not strongly interfere with our previous efforts to determine the heat of transition in cesium chloride.[1] Thus by using equations 1, 4, 5 and 6, a heat of transition around 1.4 kcal./mole is found, as compared with the value 1.55 kcal./mole based on equations 1, 2, 3, 4.[1] The calculated heat of transition is seen to deviate from the experimental value (0.58 kcal./mole) far more than can possibly be explained by a non-ideal mixing of cesium and rubidium atoms.

Data for an accurate evaluation of the remaining correction term (the last term in equation 1), which takes care of the difference in specific heat of high and low cesium chloride, do not exist. When $T_{\mathrm{tr}} - T_{\mathrm{m}}$ is small, however, this term is quite negligible (of the order of magnitude $\Delta c_{\mathrm{p}}(T_{\mathrm{tr}} - T_{\mathrm{m}})^2/2T_{\mathrm{m}}$). From the heat content curves,[3] Δc_{p} is calculated to be 0.173 cal./mole deg. at 745°K., a value which entirely justifies the exclusion of this term from the calculations. Actually an agreement between the observed heat of transition and the heat of transition calculated from the phase diagram cannot be obtained by choosing any reasonable function for Δc_{p}. Therefore (provided that only a negligible amount of rubidium chloride is soluble in low cesium chloride at any temperature) the experimental results appear to be inconsistent.

SVENSKA SILIKATFORSKNINGSINSTITUTET
GÖTEBORG, SWEDEN J. KROGH-MOE

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THE n → π* TRANSITION IN NUCLEIC ACIDS AND POLYNUCLEOTIDES

Sir:

The 2600 Å. absorption band of the nucleic acids is often tacitly assumed to be a single electronic band. However, spectroscopic studies suggest that the seemingly simple near-ultraviolet band is actually rather complex, aside from the superposition of pyrimidine and purine bands. First it is clear[1] that the purine absorption band consists of two independent and mutually perpendicularly polarized $\pi \rightarrow \pi^*$ transitions, involving the conjugation electrons of both rings. Secondly, both in the pyrimidines[2] and purines[2–4] there is an $n \rightarrow \pi^*$ transition region on the long wave length side of the strong near ultraviolet $\pi \rightarrow \pi^*$ absorption band, even in aqueous solution. The $n \rightarrow \pi^*$ absorption appears in the N-heterocyclic bases in aqueous solution often as merely a shoulder or poorly resolved inflection in the absorption curve.

The $n \rightarrow \pi^*$ transitions originate in the promotion of a non-bonding (n-orbital) lone-pair electron on a hetero-atom (O or N) to an empty π-antibonding molecular orbital. The $n \rightarrow \pi^*$ transitions are distinguished from $\pi \rightarrow \pi^*$ transitions by several inherent characteristics, of which two are of special interest here: (a) the $n \rightarrow \pi^*$ absorption bands are of intrinsically low intensity, being at most one-tenth as intense as the $\pi \rightarrow \pi^*$ bands; (b) the $n \rightarrow \pi^*$ bands have transition moments polarized out-of-plane, whereas the $\pi \rightarrow \pi^*$ transition moments are polarized in-plane for all pyrimidines and purines. Thus for DNA or other two stranded helical polynucleotides, the transition moment for the $\pi \rightarrow \pi^*$ transition is perpendicular to the molecular helix axis, while the $n \rightarrow \pi^*$ transition moment is parallel to the helix axis.

Dichroic studies on oriented polymer films should reveal the characteristic differences in polarization directly. Accordingly, a series of ultraviolet absorption curves in polarized light were measured using oriented films of various synthetic polynucleotides and nucleic acids. Films were prepared by placing the polymer sample on a quartz disk and dissolving the material in a small droplet of water. This produced a gel which was stroked until the material had air dried. The films were optically clear, and examination under the polarizing microscope revealed several areas of high birefringence. The disks were placed in a Cary Model 14 recording spectrophotometer which had been fitted with Glan Thompson prisms so that the polarized ab-

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(4) M. Kasha, in "The Nature and Significance of $n \rightarrow \pi^*$ Transitions," "Symposium on Light and Life," Johns Hopkins University Press, Baltimore, Md., 1960.