TABLE I

INCORPORATION OF LABELED PYROPHOSPHATE INTO RIBONUCLEOSIDE TRIPHOSPHATES

0.1M tris pH 7.5, 0.1M KF, 0.01M $MgCl_2$, 0.005M PP_i (75,000 c.p.m. in A; 41,000 c.p.m. in B, 150,000 c.p.m. in C). In A, 50–100% $(NH_4)_2SO_4$ cut of embryonic heart supernatant (S_H) (4.1 mg. protein), 120 γ of S-RNA. In B, 3.125 μmoles ATP, 0.025 μmole each of GTP, CTP and UTP per ml.; enzyme: a 60–95% $(NH_4)_2SO_4$ cut of 14 day embryonic liver supernatant (S_L), (3.3 mg. protein), S-RNA as in A. In C nucleotide mixture of B; mixed soluble and microsomal fractions of 14 day chick liver, no RNA added.

Expt.	Material added	Amount	Duration min.	C.p.m. incorporated[a]
A	Mixture of triphosphates	0.5 μmole total (0.125 μmole each)	5	1745
	ATP	0.50 μmole	5	2385
	GTP	0.50 μmole	5	1295
	CTP	0.50 μmole	5	1200
	UTP	0.50 μmole	5	1935
B	S-RNA	None	20	260
	S-RNA	12γ	20	648
	S-RNA	57γ	20	1876
	S-RNA	232γ	20	2656
	S-RNA	348γ	20	3456
C	Mixture of triphosphates	0.50 μmole total	30	2960
	Mixture of diphosphates	0.50 μmole total	30	688
	Triphosphates + 18 amino acids	0.50 μmole total	30	0

TABLE II

INCORPORATION OF ATP-8-C^{14} INTO POLYMERIC MATERIAL

10 μmoles tris pH 7.5, 5 μmoles $MgCl_2$, 0.026 μmole ATP-8-C^{14} (Schwarz Labs. 1.926 $\times$ 10^5 c.p.m.), 0.25 μmole each of GTP, CTP and UTP. In exp. F 2.3 mg. of 60–100% $(NH_4)_2SO_4$ cut of S_H, in G crude enzyme freed of RNA by prior pyrophosphorolysis.

Expt.	Reaction mixture	S-RNA	Duration, min.	C.p.m. incorporated
F	Complete	None	10	145
	Complete	120γ	5	253
	Complete	120γ	10	473
	Complete	120γ	20	760
G	Complete	None	20	40
	Complete	120γ	20	138
	ATP-C^{14} only	120γ	20	15
	Complete	420γ	20	200
	ATP-C^{14} only	420γ	20	304

Soluble, crude enzyme preparations, from the non-sedimentable fraction of homogenates of embryonic chick hearts and livers catalyze the following three reactions: (1) the incorporation of PP_i^{32} into ribonucleoside triphosphates; (2) the pyrophosphorolysis of S-RNA, a polyribonucleotide obtained from the non-sedimentable fraction of homogenates of embryonic liver by a phenol method[9,12]; (3) the incorporation of ATP-8-C^{14} into an $HClO_4$-insoluble, presumably polymeric fraction: Table I indicates that reaction 1 specifically requires the presence of ribonucleoside 5′-triphosphates either singly or in combination; if a more highly purified enzyme preparation, low in endogenous RNA, is used the reaction becomes dependent on added S-RNA. Reaction 2 occurs with PP_i but not with P_i, and appears to favor S-RNA over other polyribonucleotides.[13] Table II indicates that reaction (3) requires either the presence of a mixture of ribonucleoside triphosphates, or of polyribonucleotides, presumably as a source of the latter. The relation of the three activities to each other and to the net synthesis of polyribonucleotide is under investigation.

DEPARTMENT OF CHEMISTRY
INDIANA UNIVERSITY
BLOOMINGTON, INDIANA
C. W. CHUNG
H. R. MAHLER

RECEIVED APRIL 19, 1958

(10) E. Herbert, V. R. Potter and L. I. Hecht, *J. Biol. Chem.*, **225**, 659 (1957).

(11) M. Edmonds and R. Abrams, *Biochim. Biophys. Acta*, **26**, 226 (1957).

(12) K. S. Kirby, *Biochem. J.*, **64**, 405 (1956).

(13) ATP has been identified as a product in this reaction.

THE HEXOSAMINE MOIETY OF N-ACETYLNEURAMINIC ACID (SIALIC ACID)

Sir:

As previously reported,[1] an enzyme obtained from *Clostridium perfringens* (N-acetylneuraminic acid aldolase; NANaldolase) catalyzes the following reversible reaction: N-acetylneuraminic acid = pyruvate + N-acetyl-D-mannosamine (N-AcMm). In contrast to these results, data from other laboratories[2-4] indicated that the hexosamine moiety of N-acetylneuraminic acid (NANA) is N-acetyl-D-glucosamine (N-AcGm). The present studies suggest that the apparent discrepancy in the results obtained by the enzymatic[1] and chemical[2-4] techniques is due to the interconversion of N-AcGm and N-AcMm under the alkaline conditions used for the chemical work.

Treatment of *either* N-AcGm or N-AcMm with pyridine and nickelous acetate under the conditions used for the degradation of NANA[2] gave a mixture of N-AcGm and N-AcMm (approximately 8:2) as well as traces of unidentified components which were apparent by paper chromatography, but were not identified. Thus, 36 mg. of synthetic N-AcMm,[1] 2 ml. of anhydrous pyridine, 0.12 g. of nickelous acetate, heated at 100° for 1.5 hr. yielded 6 mg. of first crop crystalline material. The crystals were identified as N-AcGm by: (1) m.p. (202–203°, uncor., dec.; no depression on admix-

(1) D. G. Comb and S. Roseman, THIS JOURNAL, **80**, 497 (1958).

(2) R. Kuhn and R. Brossmer, *Chem. Ber.*, **89**, 2471 (1956).

(3) F. Zilliken and M. C. Glick, *Naturwissenschaften*, **43**, 536 (1956).

(4) J. W. Cornforth, M. E. Firth and A. Gottschalk, *Biochem. J.*, **68**, 57 (1958).

ture with authentic material); (2) paper chromatography,[1] which showed it to be N-AcGm free of N-AcMm; (3) an X-ray powder diffraction pattern which was identical to that obtained with N-AcGm.

NANA has been obtained by treatment of N-AcGm with oxaloacetate at *p*H 11.0.[4] A solution of N-AcGm (2.5 g. in 10 ml. of water) was therefore adjusted to, and maintained, at *p*H 11.0 at room temperature. Analysis of aliquots (Table I) at 24, 48 and 72 hr. indicated that a substantial amount of N-AcMm was formed during the first day.

TABLE I

ALKALINE EPIMERIZATION[a] OF N-AcGm TO N-AcMm

Compound	Time, hr.	$R_{N\text{-}AcGm}$	N-AcMm formed,[b] %
N-AcGm (*p*H 11.0)	0	1.0	0
	24	1.0, 0.4	19
	48	1.0, 0.4	24
	72	1.0, 0.4	24

[a] Descending chromatography was conducted on borate treated paper with butanol–pyridine–water (6:4:3) for 20 hr. Synthetic N-AcMm exhibits $R_{N\text{-}AcGm}$ 0.4 under these conditions. [b] Estimated enzymatically with NANaldolase and pyruvate.[1] At equilibrium, 10% of the added N-AcMm is converted to NANA, the latter compound determined by the direct Ehrlich reaction. No inhibition of the enzyme was noted with artificial mixtures containing N-AcGm and N-AcMm (5:1).

After 48 hr., 1.95 g. of N-AcGm (m.p. 202–203°) was recovered by fractional crystallization from ethanol. The remaining mixture (0.5 g.) contained about 90% N-AcMm, 10% N-AcGm, and traces of unidentified materials similar to those obtained in the pyridine epimerization. The N-AcMm was identified by: (1) paper chromatography[1]; (2) conversion to the expected quantity of NANA by the addition of pyruvate and NANaldolase; (3) conversion to D-mannosamine, isolated as the crystalline hydrochloride, and identified by ion-exchange chromatography, optical rotation, color reactions and its X-ray powder diffraction pattern. The alkaline epimerization of N-AcGm to N-AcMm may be a suitable method for the preparation of N-AcMm and of D-mannosamine.

The data outlined above indicate that reversible epimerizations of N-AcMm and N-AcGm readily occurs under the conditions which other investigators have used for chemical studies on the structure of NANA. In view of the present findings, and of our previous results,[1] it is concluded that the N-acetylhexosamine moiety of NANA is N-acetyl-D-mannosamine.

(5) Postdoctoral fellow, American Cancer Society.

THE RACKHAM ARTHRITIS RESEARCH UNIT AND
DEPARTMENT OF BIOLOGICAL CHEMISTRY SAUL ROSEMAN
UNIVERSITY OF MICHIGAN DONALD G. COMB[5]
ANN ARBOR, MICHIGAN

RECEIVED APRIL 14, 1958

QUANTITATIVE SPECTROMETRY OF AQUEOUS NEPTUNIUM IONS AT ELEVATED TEMPERATURES AND PRESSURES

Sir:

There are reported herein the results of what are believed to be the first quantitative spectral measurements of a liquid phase above its normal boiling point. A solution of neptunyl ion (7.43×10^{-4} *M* $RbNp^{237}O_2(NO_3)_3$ in 0.1 *M* DNO_3–D_2O at 25°)[1] has been examined from 0.6 to 1.3 μ for 45 hours and from 4.5 to 252°. Deuterated solutions were used to decrease both the absorbance and the effect of temperature upon the absorbance of the solvent.[2] The sample was saturated with ozone, then degassed prior to introduction into the pretreated titanium cell assembly. As the temperature was raised Np(VI) was found to be reduced to Np(V) quantitatively and irreversibly.

The sharp, strong 0.98 μ absorption band of Np(V) is slightly asymmetric, the integrated absorbance of the long wave length side being 1–2% greater than the short wave length side, both at 25 and 251°. The position of the maximum shifts, at a decreasing rate, from 9797 Å. at 25° to 9741 Å. at 251°. The half intensity band width shows a striking behavior with increasing temperature, first decreasing (12%) from 60 cm.$^{-1}$ at 25° to a minimum of 53 cm.$^{-1}$ at about 175°, then slowly increasing to 57 cm.$^{-1}$ at 251°. The product of the absorbance at the band maximum and the half intensity band width, normalized for solvent background and density change, decreases linearly (34%) from 25 to 251°.

From a chemical point of view the above observations suggest an effect of temperature upon the solvation sphere of the NpO_2^+ ion, and a concomitant interaction of the NO_3^- ion present with the NpO_2^+–solvate dipole system.

Several weaker absorption bands belonging to Np(V) and Np(VI) ions have been studied in less detail. The results do indicate clearly, however, a great variability in the effect of temperature upon band profile.

Expansion and refinement of measurements similar to those reported should have manifold implications toward a better understanding of the nature of ions in solution, and forces operative in the liquid state.

The equipment, which has been designed and built at this Laboratory for use with the Cary Model 14 Spectrophotometer, has a working range of 0 to 250° and 0 to 1000 p.s.i. The cell space is 7.62 cm. long × 1.00 cm. diameter, and is fabricated from annealed titanium with colorless synthetic sapphire windows. The entire sample cell and reservoir assembly is suspended in an evacuated chamber, and positioned in a special fused quartz mount. The sample cell at any operating temperature is measured optically against a geometrically similar solvent cell which is thermostated at 25°. Stability and reproducibility of measurements over the design range are comparable to that of operation at room temperature.

A complete discussion of the above results and equipment will be published in articles now in preparation.

I wish to express my appreciation to Dr. R. W.

(1) The author is indebted to Messrs. P. M. Lantz and G. W. Parker, Chemistry Division, Oak Ridge National Laboratory, for supplying a single crystal of the Np salt, and to Dr. B. M. Benjamin, also of this Laboratory, for the necessary DNO_3 stock solution.

(2) W. C. Waggener, *J. Phys. Chem.*, **62**, 382 (1958); *Anal. Chem.*, in press.