COMMUNICATIONS TO THE EDITOR

NEPTUNIUM(VI) IN MOLTEN NITRATES[1]

Sir:

There has been no direct evidence for the existence of neptunium(VI) in any molten salt system. In molten chlorides,[2] neptunium has been prepared in the (III), (IV) and (V) states. In molten nitrates,[2] the $LiNO_3$–KNO_3 eutectic, only Np(V) has been observed. A study of the solvent extraction[3] of neptunium from the $LiNO_3$–KNO_3 eutectic showed a large increase in the distribution coefficient upon the addition of NH_4NO_3. This fact could be explained by either a "salting effect" or by a valence change. This work has shown that Np(V) is oxidized to Np(VI) by the action of NH_4NO_3 in the $LiNO_3$–KNO_3 eutectic.

The reaction vessel was fabricated from a 10-mm. quartz absorption cell which was fused to a 20-cm. length of 12 mm. quartz tubing. This cell fitted into a small furnace[4] which fitted into the cell compartment of a Cary Recording Spectrophotometer Model 14. The spectral measurements in the molten nitrates were carried out at 182°.

Neptunium-237 in dilute HNO_3 was placed in the reaction vessel and carefully dried by evacuating and heating. Filtered, molten $LiNO_3$-KNO_3 eutectic was added to the cell and the resulting green solution yielded the spectrum of Np(V). The addition of NH_4NO_3 to the melt resulted in bubbling and spectral measurements showed the Np(V) peak at 9820 Å. to decrease and a new peak at 11,600 Å. to grow in. The next day the Np(V) peak had decreased to only 2% of its original value. The color of the melt changed from bright green to a brownish yellow.

Since the major peak of Np(VI) in perchlorate solution is at 12,230 Å.,[5] the spectrum of Np(VI) in concentrated nitric acid was measured. In this case the major peak occurred at a still shorter wave length, 11,220 Å.

The action of NH_4NO_3 in molten nitrates is extremely interesting. For instance, NpO_2 is insoluble, in molten nitrates. However, the addition of NH_4NO_3 to the melt at a temperature of 250° will dissolve the oxide quickly to yield Np(V) in solution. UO_3, which is insoluble in molten nitrates, is dissolved easily by the action of NH_4NO_3. NH_4NO_3 dissolves many metallic oxides in the molten nitrate system.[6] It acts as both an acid and an oxidizing agent in molten nitrates.

The Np(VI) ion is not stable in the nitrate melt and in a matter of days this ion will revert to the (V) state. Also the Np(VI) ion is not stable with respect to higher temperatures. At about 220° the Np(VI) is reduced to Np(V). The Np(V) ion is stable in nitrate melts to about 350°. At 380° neptunium precipitates from the solution and as yet the brown solid has not been identified; however, it probably is NpO_2.

The oxidation of Np(V) to Np(VI) also may be accomplished by bubbling ozone through the solution of Np(V) in the nitrate melt. Sometimes during this oxidation a brown precipitate forms. Work is now in progress to identify this solid.

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(1) Based on work performed under the auspices of the U. S. Atomic Energy Commission.

(2) D. M. Gruen, S. Fried, P. Graf and R. L. McBeth, "The Chemistry of Fused Salts," U. N. Peaceful Uses of Atomic Energy, Proceedings of the Second International Conference, Geneva, Sept., 1958, Vol. 28, paper P/940.

(3) N. M. Isaac, P. R. Fields and D. M. Gruen, paper given at 138th meeting ACS, New York, N. Y., "Solvent Extraction of Actinides and Lanthanides from Molten Salts."

(4) J. P. Young and J. C. White, *Anal. Chem.*, **31**, 1892 (1959).

(5) W. C. Waggener, *J. Phys. Chem.*, **62**, 382 (1958).

(6) This observation has also been reported by N. M. Isaac, P. R. Fields and D. M. Gruen, *J. Inorg. Nuclear Chem.*, in press.

A METHYLATED DERIVATIVE OF TETRAHYDROFOLATE AS AN INTERMEDIATE OF METHIONINE BIOSYNTHESIS[1]

Sir:

In a recent communication[4] a report has been made of an enzymatic system isolated from a mutant[5] of *Escherichia coli* (113-3, Davis, grown on vitamin B_{12} and methionine) which is capable of carrying out the reaction

$$N^5,N^{10}\text{-methylene folate-H}_4^6 + \text{homocysteine} \longrightarrow \text{methionine} + \text{folate-H}_4$$

This enzymatic system is comprised of several cofactors (DPNH, FAD, ATP, Mg^{++}) and two enzymes, which have now been partially purified. One enzyme is the component missing in another

(1) This work was supported by a grant-in-aid from the National Science Foundation. Brief reports of the isolation of an intermediate of methionine biosynthesis have been made by this laboratory[2] and by Wilmanns, *et al.*[3]

(2) A. R. Larrabee and J. M. Buchanan, *Federation Proc.*, **20**, 9 (1961).

(3) W. Wilmanns, B. Rücker and L. Jaenicke, *Z. physiol. Chem.*, **322**, 283 (1960).

(4) F. T. Hatch, A. R. Larrabee, R. E. Cathou and J. M. Buchanan, *J. Biol. Chem.*, **236**, 1095 (1961).

(5) B. D. Davis and E. S. Mingioli, *J. Bacteriol.*, **60**, 17 (1950).

(6) Abbreviations used are: folate-H_4, tetrahydrofolate; DPNH, diphosphopyridine nucleotide (reduced form); FAD, flavin adenine dinucleotide; ATP, adenosine triphosphate.

(7) F. Pregl and J. Grant, "Quantitative Organic Microanalysis," The Blakiston Company, Philadelphia, Pa.

(8) This observation was made by Dr. Victor Herbert.

(9) R_f = 0.31 in 0.1 M phosphate buffer, pH 7.0.

(10) ADDENDUM.—Since this communication was submitted for publication a paper by Keresztesy and Donaldson has appeared which describes some recent studies on prefolic A, a compound isolated by them from horse liver. In consultation with these authors at the Federation Meetings in April 1961 several properties of their compound resembled those of the intermediate of methionine biosynthesis. N^5-methyl folate-H_4, reported by us at that time. Keresztesy and Donaldson[11] have now synthesized this compound (as have Sakami and Ukstins[12]) by reduction of N^5,N^{10}-methylene folate-H_4 with borohydride and have shown that it may be converted to formaldehyde and folate-H_4 by their enzymatic system. We have now prepared this compound synthetically by their method and have found that it contains one mole of methyl group per mole of folate compound. It is converted in our enzymatic system to methionine but to only one-half the extent of the enzymatically prepared compound.

(11) J. C. Keresztesy and K. O. Donaldson, *Biochem. and Biophys. Res. Comm.*, **5**, 286, 289 (1961).

(12) W. Sakami and I. Ukstins, *J. Biol. Chem.*, **236**, PC50 (1961).

mutant of *E. coli* (205–2, Davis) and has been designated as the "205–2" enzyme. The other enzyme contains vitamin B_{12} or a derivative as a prosthetic group and has been designated as the "B_{12}-enzyme." The purpose of this communication is to report the order of action of these two enzymes and to describe an intermediate of the above reaction.

When "205–2" enzyme was incubated with DPNH and N^5,N^{10}-methylene folate-H_4 (labeled in the methylene carbon with C^{14}), a radioactive product was formed which upon acid hydrolysis yielded one mole of glutamate per mole of the carbon atom labeled with C^{14}. This product could be isolated by application to a triethylaminoethyl cellulose column (3.8 cm. in diameter × 22 cm. in height) and by elution with 0.1 *M* ammonium carbonate. The elution of the product was followed by its radioactivity and emerged from the column between 5 and 9 column volumes. A spectrum was taken of a sample eluted at the peak of the curve of radioactivity and represents the absorption of compound (compound I) which had undergone a minimum of oxidative side reactions (Curve A, Fig. 1). The pooled radioactive fractions then were lyophilized at 4° until most of the ammonium carbonate was removed. The residue was taken up in a small volume of 0.02 *M* ammonium carbonate without undue agitation or exposure to air. The spectrum of an aliquot of this material (not shown in Fig. 1) agreed with that of compound I except that the absorption in the region ot 250 mμ was slightly higher than might be expected from the absorption at 290 mμ used as a reference point. Thus, a small but measurable amount of oxidation of the compound probably had taken place. Compound I, which is a folate derivative, has one symmetrical absorption peak with a maximum at a wave length of 290 mμ (*cf.* ref. 3). Based on radioactivity, the intermediate has a molar absorbancy index of about 29×10^6 mole^{-1}cm.^{2}. When this material is shaken in air or oxygen at *p*H 8.7 it is changed to a second compound (Curve B, Fig. 1) which has absorption maxima at 290 and 250 mμ. The ratio of light absorption at these two wave lengths is about 1.28. The spectrum does not change with further exposure to oxygen at this pH. If the pH of the solution containing compound II is changed to 4.3, a third spectrophotometric species (Compound III, Curve C, Fig. 1) is formed which has an absorption maximum at 282 mμ. In the presence of PtO_2 and H_2 at pH 8 Compound II may be reduced with the uptake of one mole of H_2 to yield a material with the spectrum of Compound I. Compound I does not react with the aldehyde binding reagent, 5,5-dimethyl-1,3-cyclohexanedione (dimedon), and upon treatment with HI yields radioactive CH_3I which has been isolated as the quaternary iodide.[7] It serves as a growth factor for *Lactobacillus casei*[8] but not for *Streptococcus faecalis* or *Leuconostoc citrovorum*.

At the pH of the enzymatic incubation, compounds I and II may serve as substrates in the second reaction and both require homocysteine, DPNH, FAD, Mg^{++}, ATP and "B_{12}-enzyme." The products of the reaction were methionine and folate-H_4. The latter was identified by its ability to support growth of *L. citrovorum* and by its migration index (R_f) in paper chromatography.[9] N^{10}-Methyl folate-H_4 prepared by the catalytic reduction of N^{10}-methyl folate could not serve as a substrate of the second reaction.

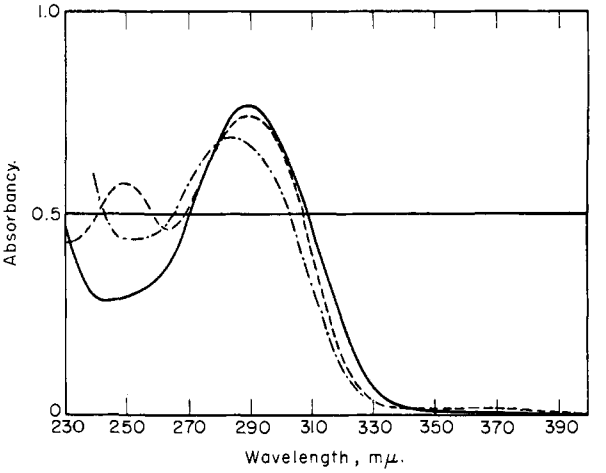

Fig. 1.—Absorption spectra of methyl tetrahydrofolate and oxidized derivatives: Curve A——, Compound I; Curve B - - -, Compound II; Curve C—·—·, Compound III. The concentration of folate derivatives was $2.66 \times 10^{-5}M$.

From these data we wish to propose N^5-methyl tetrahydrofolate as the tentative structure of compound I. On the basis of the hydrogenation experiments, compound II would be an N^5-methyl dihydrofolate and compound III, another partially oxidized form of compound I.[10]

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THE USE OF A PROTON-PROTON SPIN DECOUPLING METHOD FOR THE DETERMINATION OF NUCLEAR MAGNETIC RESONANCE CHEMICAL SHIFTS

Sir:

The complexities of high-resolution n.m.r. spectra of molecules can in many cases be simplified drastically by the use of double resonance spin decoupling techniques.[1] Until recently the application of spin decoupling techniques to proton–proton systems was limited by the complexity of the required instrumentation.[2] A new technique for accomplishing proton–proton spin decoupling with relatively simple instrumentation has been described recently by Kaiser[3] and Freeman[4]; this is the audio side band phase detection technique.[5] We now describe a new application of

(1) See J. A. Pople, W. G. Schneider and H. J. Bernstein, "High-resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., New York, N. Y., 1959, pp. 160–161, 229–230, 298–305 and 370–371, for discussions and leading references.
(2) W. A. Anderson, *Phys. Rev.*, **102**, 151 (1956).
(3) R. Kaiser, *Rev. Sci. Instr.*, **31**, 963 (1960).
(4) R. Freeman, *Molecular Phys.*, **3**, 435 (1960).
(5) J. Itoh and S. Sato, *J. Phys. Soc. Japan*, **14**, 851 (1959), previously described an audio side band technique without phase detection;