phase was thought, without proof of composition, to be $(PN)_n$. We have found that the crystalline phase produced under the conditions described, and under other conditions, is $P_3N_5$.

The solid phosphorus nitrides produced by use of electrical discharges are amorphous to X-rays and usually are heterogeneous in appearance. They decompose at 800 to 900° in a vacuum or in ammonia or nitrogen. The volatile products are mainly the elements; under suitable conditions, a homogeneous amorphous nitride with an N:P atomic ratio of unity can be condensed therefrom. The properties of the residual solid depend upon the starting material and its treatment.

Effects of several treatments on various amorphous nitrides are shown in Table I. The heterogeneous amorphous mixtures (products of arc synthesis) invariably yielded crystalline residues with the same X-ray pattern, and the intensity of the pattern increased as the N:P ratio approached 1.66. The conversion of amorphous to crystalline phase was accompanied by an expansion.

TABLE I

REACTIONS OF AMORPHOUS NITRIDES IN VACUUM, AMMONIA AND NITROGEN AT 800 TO 900°

| Initial material (amorphous) | | Residual product | |
|---|---|---|---|
| Appearance[a] | Atomic ratio N:P | Atomic ratio N:P | Crystallinity[b] |
| In vacuum, $10^{-3}$ to $10^{-4}$ mm. | | | |
| Homogeneous | 0.98 | 0.98 | Absent |
| | 1.48 | 1.48 | Absent |
| Heterogeneous | 1.25 | 1.60 | Strong |
| | 1.25 | 1.65 | Very strong |
| In ammonia, 1 atm. | | | |
| Homogeneous | 0.99 | 1.62 | Very weak |
| | 1.48 | 1.58 | Very weak |
| Heterogeneous | 1.22 | 1.27 | Very weak |
| | 1.22 | 1.61 | Weak |
| | 1.22 | 1.63 | Strong |
| | 1.22 | 1.66 | Very strong |
| In nitrogen, 1 atm. | | | |
| Homogeneous | 0.99 | 1.46 | Weak |
| | 1.48 | 1.35 | Absent |
| Heterogeneous | 1.26 | 1.56 | Medium |

[a] Under microscope. [b] All crystalline phases gave same X-ray pattern.

Homogeneous amorphous materials with N:P ratios of 1 and 1.5—corresponding to $(PN)_n$ and $(P_2N_3)_n$—reacted similarly with ammonia to yield the same crystal phase. The mononitride also reacted with nitrogen. Neither of these materials, however, changed in N:P ratio or yielded a crystalline phase when heated in a vacuum.

It was concluded that the crystalline phase was $P_3N_5$. The interplanar spacings, $d$, and the relative intensities, $I$, are shown in Table II.

TABLE II

X-RAY POWDER DIFFRACTION PATTERN OF $P_3N_5$[a] (Cu $K_\alpha$ RADIATION[a]

| $d$, Å. | $I$ | $d$, Å. | $I$ | $d$, Å. | $I$ |
|---|---|---|---|---|---|
| 4.56 | S | 2.02 | MW | 1.34 | W |
| 4.20 | M | 1.83 | VW | 1.29 | W |
| 3.60 | VS | 1.71 | W | 1.21 | W |
| 2.72 | MW | 1.61 | W | 1.20 | W |
| 2.55 | VW | 1.53 | M | 1.17 | VW |
| 2.43 | MW | 1.50 | W | 1.12 | W |
| 2.36 | MW | 1.46 | MW | 1.08 | W |
| 2.27 | MW | 1.41 | W | 1.04 | VW |

[a] Determined by James P. Smith.

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## PHOSPHORYLATION COUPLED WITH PYRIDINE NUCLEOTIDE OXIDATION[1]

*Sir:*

We are reporting herewith mitochondrial phosphorylations in beef heart and honey bee thoracic preparations. These phosphorylations are coupled to oxidation of triphosphopyridine nucleotide (TPNH), and also depend upon an enzyme or enzymes present in the "soluble" fraction of the cells, *i.e.*, the portion not sedimented by centrifuging up to 105,000 × *g*.

Table I records the oxidation and phosphorylation observed when TPNH is the substrate. Results are similar with catalytic amounts of TPN in the glucose-6-$PO_4$ or 6-phosphogluconate oxidation systems. It is evident that the "soluble" fraction, which contains the pentose cycle complex,[2] does not possess the necessary terminal electron transport enzymes to promote TPNH and pentose cycle oxidations. On the other hand, both the "soluble" fraction and the mitochondria are required for oxidative phosphorylation, in contrast to Krebs cycle oxidations where the mitochondria alone can suffice. Freshly prepared sarcosomes from honey bee thoraces also oxidize TPNH with concomitant phosphorylation, but do not require a "soluble" fraction. The P/O ratios in the honey bee are low (0.2) both with TPNH and Krebs cycle intermediates because of an active ATPase in the preparations.

Against the possibility that the phosphorylations may have been due to glycolysis (from ADP-myokinase-glucose-hexokinase), additional experiments were carried out using glucose-$C^{14}$ and inhibitors. By using glucose-1-$C^{14}$ or glucose-6-$C^{14}$ in the presence or absence of iodoacetate it was found that the phosphorylation associated with TPNH oxidation was not due to glycolysis. Similar P/O ratios were observed during oxidation of DPNH. This may be due at least in part to glycolysis.

The simultaneous requirement for both the "soluble" and particulate fraction to promote oxidative phosphorylation with TPNH is observed either with fresh or frozen and thawed preparations. While problems of mitochondrial permeability to TPN and TPNH remain to be settled,

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(2) R. W. Newburgh and V. H. Cheldelin, *ibid.*, **218**, 89 (1956).

TABLE I

OXIDATIVE PHOSPHORYLATION BY BEEF HEART MITOCHONDRIA AND SOLUBLE PREPARATION

Each vessel contained 27.2 μmoles of phosphate buffer, *p*H 7.4, containing $P^{32}$ (1187 c.p.m./μmole phosphorus), 20 μmoles of $MgCl_2$, 10 μmoles of ADP, 50 μmoles of glucose, 6 mg. of hexokinase; and where indicated, 20 μmoles of pyruvate, 5 μmoles of malate, 10 μmoles of TPNH; 0.3 ml. of mitochondria, 0.5 ml. of $S_{25,000}$ enzyme fraction as indicated. Final volume 3.0 ml., incubated 15 minutes at 30°. The reaction was stopped by adding 0.2 ml. of 3 *M* TCA. Inorganic phosphate was measured colorimetrically using a 0.1-ml. aliquot. Differences from the control of 0.3–0.4 optical density units were found, where $\Delta$O.D. of 0.20 represents 0.27 μmole of P. Esterified phosphorus was measured using the extraction procedure of Cooper and Lehninger.[3] The amount of phosphorus esterified agreed by the two methods used. The mitochondria were prepared by the method of Hogeboom and Schneider[4] and used after storing for 24 hours at −10°. The $S_{25,000}$ fraction was the supernatant layer obtained after centrifuging a cell homogenate at 0° for three hours at 25,000 × *g*, in two volumes of medium containing 0.9% KCl and 0.001 *M* ethylenediamine tetraacetate ("Versene"). The supernatant fraction was dialyzed against 2 liters of 0.02 *M* tris buffer, *p*H 7.4 for 12 hours. In other experiments not shown, the $S_{105,000}$ fraction was employed with similar results.

| Enzyme added | Substrate | μatoms oxygen consumed Expt. I | μatoms oxygen consumed Expt. II | μatoms P esterified Expt. I | μatoms P esterified Expt. II | P/O Expt. I | P/O Expt. II |
|---|---|---|---|---|---|---|---|
| Mitochondria | Pyruvate + malate | 11.8 | 14.1 | 23.3 | 24.8 | 2.0 | 1.8 |
| Mitochondria + $S_{25,000}$ | Pyruvate + malate | 10.7 | 11 | 24.4 | 25.2 | 2.3 | 2.3 |
| $S_{25,000}$ | Pyruvate + malate | 0 | ... | 0 | ... | 0 | ... |
| Mitochondria | TPNH | 5.4 | 10.7 | 0.6 | 2.0 | 0.1 | 0.2 |
| Mitochondria + $S_{25,000}$ | TPNH | 5.9 | 9.1 | 9.7 | 18.9 | 1.6 | 2.1 |
| $S_{25,000}$ | TPNH | 0 | ... | 0 | ... | 0 | ... |

the present findings may suggest an *in vivo* cooperation between mitochondria and "soluble" enzymes, that could permit utilization of energy from such systems as the pentose cycle.

(3) C. Cooper and A. L. Lehninger, *J. Biol. Chem.*, **219**, 489 (1956).
(4) G. H. Hogeboom and W. C. Schneider, *ibid.*, **194**, 513 (1952).
(5) Postdoctoral Fellow of the United States Public Health Service.

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## OXIDATION OF CYCLOÖCTATETRAENE TO THE TROPYLIUM CATION

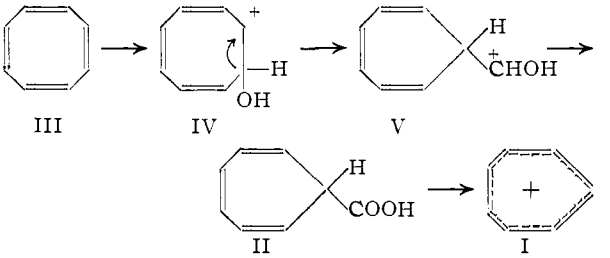
*Sir:*

In a previous paper[1] it was stated that the tropylium cation (I) was oxidized readily by permanganate to benzaldehyde. During subsequent experiments, designed to see whether this reaction could be used as a specific test for the presence of small amounts of tropylium salts in aqueous solution, we have found that norcaradiene-carboxylic acid (II)[2] is similarly readily oxidized to benzaldehyde. The tropylium cation probably is formed as an intermediate in the latter reaction for we have been able to isolate tropylium salts in yields of up to 30% from the reaction mixture. We have obtained similar results,[3] though with varying yields of tropylium salts, following the oxidation of norcaradiene-carboxylic acid with other oxidants, *e.g.*, ceric salts, $Pb(OAc)_4$, $HIO_4$, $Na_2S_2O_8$, and we consider that a common two-electron transfer mechanism operates in most of these reactions.

Other oxidation products of norcaradiene-carboxylic acid with permanganate are benzoic acid and benzaldehyde. Terephthalic acid also was obtained when chromic acid in acetic acid was used as the oxidant. There appears to be a close parallel between these results and the oxidation products of cycloöctatetraene (III). Reppe and co-workers report[4] that neutral permanganate oxidizes III to benzoic acid and benzaldehyde, while chromic acid oxidizes it to benzaldehyde and terephthalic acid. It seemed possible therefore that norcaradiene-carboxylic acid might be formed as an intermediate in the oxidation of cycloöctatetraene and, in view of the results described above, tropylium salts also could be formed.[5]

We have now found that appreciable yields (5%) of tropylium salts can be isolated from the reaction products following the oxidation of cycloöctatetraene with permanganate in dilute sulfuric acid–acetone mixtures. To isolate the tropylium salt it is necessary to first extract the other neutral and acidic products of the reaction, then convert the water soluble tropylium sulfate to ditropyl ether, which is then extracted and converted to tropylium bromide with HBr. This method certainly involves losses and the figure of 5% is the minimum yield of tropylium sulfate formed in the reaction. When the oxidation is performed under neutral or alkaline conditions the yield of tropylium salt is negligible.

We consider that the formula scheme shown adequately accounts for the formation of the tropylium cation in this reaction.




The first step is the formation of a glycol which, in the acid solution used, gives rise to the ion IV. This then undergoes the normal pinacol–pinacolone rearrangement to give the cation V, which is pro-

(1) M. J. S. Dewar and R. Pettit, *J. Chem. Soc.*, 2026 (1956).
(2) The absolute structure of norcaradiene-carboxylic acid is not yet established definitely.[3] A 1,3,5-cycloheptatriene formulation is used in this paper.
(3) Full details to be published elsewhere.
(4) W. Reppe, *et al.*, *Ann.*, **560**, 1 (1948).
(5) W. von E. Doering, *et al.*, THIS JOURNAL, **78**, 5448 (1956).