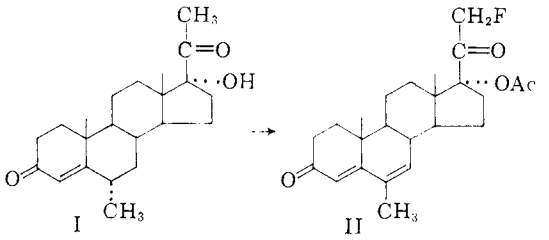

I II

fluorine containing steroid was acetylated with acetic anhydride, *p*-toluenesulfonic acid,[5] then treated with methanol and hydrochloric acid to hydrolyze any enol acetate that may have formed. The resulting material was chromatographed on silica gel. By the rechromatography of the crystalline steroid eluted with 10% ethyl acetate in benzene, there was obtained 6-methyl-17α-acetoxy-21-fluoro-4,6-pregnadiene-3,20-dione (II)[6]; m.p. 222-223°; $[\alpha]_D$ −2.5° ($CHCl_3$); $\lambda_{max}^{methanol}$ 288 mμ, (ϵ 23,300); λ_{max}^{KBr} 5.73, 5.98, 6.13, 6.31, 7.88 and 8.05 μ; (found: C, 71.78; H, 7.91).

When tested orally in the Clauberg assay[7] at a level producing a +2 degree of glandular arborization, compound II was 17 times as potent as subcutaneous progesterone or 1700 times as potent as oral progesterone. It was three times as potent orally as 6α-methyl-17α-acetoxyprogesterone.[2b]

(5) R. B. Turner, THIS JOURNAL, **75**, 3489 (1953).

(6) A. Bowers and H. J. Ringold [*ibid.*, **80**, 3091 (1958)] have treated 11-oxo-6α-methyl-17α-hydroxyprogesterone with iodine (2.1 moles/mole of steroid) and calcium oxide (9.6 moles/mole of steroid) in tetrahydrofuran–methanol in practically the same way as we have treated 6α-methyl-17α-hydroxyprogesterone (I), but they have not reported the formation of any 6-methyl-6-dehydrosteroid. It should be noted that the 6,7-double bond probably was introduced during the iodination. The crude iodo-compound had a maximum in the ultraviolet at 291 mμ, (ϵ 10,500).

(7) C. W. Emmens, "Hormone Assay," Academic Press, Inc., New York, N. Y., 1950, p. 422.

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THE FUNCTION OF CYTIDINE DIPHOSPHATE DIGLYCERIDE IN THE ENZYMATIC SYNTHESIS OF INOSITOL MONOPHOSPHATIDE[1]

Sir:

In previous studies of the enzymatic synthesis of inositol monophosphatide, Agranoff, Bradley and Brady[2] showed that tritiated CMP[3] could be enzymatically converted to a lipid compound, tentatively identified as CDP-diglyceride,[3] while Paulus and Kennedy[4] showed that the phosphorus moiety of inositol monophosphatide is derived from L-α-glycerophosphate and that CTP is specifically required for this reaction sequence. These findings are consistent with the occurrence of these enzymatic reactions, for which further evidence has now been obtained: (1) CTP + L-α-glycerophosphate + 2 RCO-S-CoA → → CDP-diglyceride; (2) CDP-diglyceride + inositol → inositol monophosphatide + CMP.

When Cyt-P^{32}-P-P + DL-α-glycerophosphate are incubated with an acylating system (ATP, CoA and oleic acid) in the presence of an enzyme preparation from guinea pig liver, an extensive conversion to a labeled ether-soluble nucleotide occurs (Table I). This compound does not accumulate if *myo*-inositol is added to the system, indicating the occurrence of reaction (2). The conversion of L-α-glycerophosphate to CDP-diglyceride presumably involves a series of steps, which have not yet been studied in detail, but which may involve either phosphatidic acid or CDP-glycerol as intermediates.

TABLE I

CONVERSION OF CYT-P^{32}-P-P TO CDP-DIGLYCERIDE

System: Cyt-P^{32}-P-P, 1.0 μmole (37,000 c.p.m.); DL-α-glycerophosphate, 1.0 μmole; CoA, 0.2 μmole; oleic acid, 0.1 μmole; ATP, 5 μmoles; $MnCl_2$, 3 μmoles; $MgCl_2$, 3 μmoles; 0.5 ml. of a dialyzed whole homogenate of guinea pig liver in 0.05 *M* phosphate buffer, *p*H 7.4. The final volume was 1.5 ml. Incubation was for 1 hour at 37°. The lipids were extracted with hot methanol, transferred to ether, and an aliquot of the washed ether phase was counted.

Additions	None	1 μmole *myo*-inositol
CDP-diglyceride, mμmoles	70	7

For the direct study of reaction (2) CDP-dipalmitin was synthesized from CMP and dipalmitoyl-DL-α-glycerophosphoric acid by a method essentially similar to that used for the synthesis of CDP-choline.[5] The CDP-dipalmitin was precipitated as the barium salt from aqueous solution, dissolved in chloroform–methanol by the addition of hydrogen chloride and chromatographed on silicic acid. It was eluted at about 20% methanol in chloroform, using gradient elution. The cytidine:phosphate:ester ratio was 1.00:1.93:2.03 and the purity was estimated at 93%. The yield was 6–7%.

TABLE II

REACTION OF CDP-DIPALMITIN WITH INOSITOL

Each tube contained washed and dialyzed chicken liver microsomes in 0.5 ml. of 0.05 *M* Tris buffer *p*H 7.5 and 2 μmoles $MnCl_2$ in a total volume of 1.0 ml. and was incubated at 40° for 1 hour. The CMP released was determined spectrophotometrically at 280 mμ in the supernatant after deproteinization with perchloric acid. The lipids were extracted with hot methanol, transferred to chloroform, and an aliquot of the washed chloroform layer counted in a windowless gas-flow counter with appropriate H^3-inositol standards.

	Additions	CMP released (mμmoles)	H^3-inositol incorp. (mμmole)
1	330 mμmoles CDP-dipalmitin	0	. .
2	2 μmoles H^3-inositol	. .	4
3	330 mμmoles CDP-dipalmitin + 2 μmoles H^3-inositol	73	79

The enzymatic reaction of synthetic CDP-diglyceride with inositol, with the formation of inositol monophosphatide and the release of CMP, is shown in Table II. Preparations of microsomes from chicken liver in which the exchange reaction[4] of inositol with inositol monophosphatide is low

(1) Supported by grants from the Nutrition Foundation, Inc., the Life Insurance Medical Research Fund and the National Institute for Neurological Diseases and Blindness (B 1199). Mr. Henry Paulus is a pre-doctoral Fellow of the National Science Foundation.

(2) B. W. Agranoff, R. M. Bradley and R. O. Brady, *J. Biol. Chem.*, **233**, 1072 (1958).

(3) Abbreviations: CDP-diglyceride = cytidine diphosphate diglyceride; CMP = cytidine-5′-phosphate; CTP = cytidine-5′-triphosphate; Tris = tris-(hydroxymethyl)-aminomethane.

(4) H. Paulus and E. P. Kennedy, THIS JOURNAL, **80**, 6689 (1958).

(5) E. P. Kennedy, *J. Biol. Chem.*, **222**, 185 (1956).

in comparison with the capacity for *de novo* synthesis, were used in this experiment.

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THE UNCOUPLING OF OXIDATIVE PHOSPHORYLATION BY CARBON MONOXIDE[1]

Sir:

Experiments carried out with isolated animal mitochondria have shown that the inhibition of electron transport by agents which combine specifically with respiratory chain components, such as carbon monoxide[2] and low concentrations of azide,[3] does not significantly reduce the ratio of phosphate esterified to oxygen consumed (P/O). In contrast, we have reported[4] that low concentrations of cyanide can uncouple oxidative phosphorylation by higher plant mitochondria. Because cyanide may be involved in a variety of reactions, an attempt was made to show such an effect with a more specific inhibitor. Using mitochondria prepared from sweet potato roots, it has been possible to demonstrate a differential effect of carbon monoxide on phosphate and oxygen uptake (Table I). Whereas phosphorylation is markedly inhibited by the 4/1 CO/O_2 mixture, electron transfer is only slightly reduced. A comparison of the data for succinate and citrate suggests that a single phosphorylation step which is common to both substrates is eliminated.

The effect of carbon monoxide on phosphorylation presumably results from a specific reaction with cytochrome oxidase. This conclusion is strongly supported by the fact that it can be reversed either by increasing the oxygen concentration (substrate = citrate) or by carrying out the reaction in bright light (substrate = succinate). In addition, there was no significant carbon monoxide inhibition of the $P/2_e$ ratio when ferricyanide served as the electron acceptor under anaerobic conditions. That the uncoupling action is not due to a decrease in the rate of electron flow *per se* is clear from the fact that inhibition at the dehydrogenase level by malonate (with succinate) or $-SH$ combining agents (with citrate) did not lower the P/O ratio, and in some cases it was increased markedly.

TABLE I

Substrate	Gas phase	O_2 μatoms	P_i μatoms	P/O
Succinate	Air	15.1	18.4	1.22
	80% CO–20% O_2	12.9	5.2	0.40
Citrate	Air	13.2	26.8	2.03
	80% CO–20% O_2	13.0	16.5	1.27

The reaction mixtures (3 ml.) contained (in μmoles): substrate 60, phosphate 80, $MgSO_4$ 20, ADP 3, DPN 1.2, DPT 0.2, CoA 0.1, glucose 60, sucrose 1325, TRIS (*p*H 7.0) 12, hexokinase 1 mg., and 0.25 ml. of washed mitochondrial suspension (2 mg. protein). Reaction carried out at 30° in the dark for 30 min. Sweet potato root tissue was blended briefly in medium containing 0.5 *M* sucrose, 0.05 *M* TRIS (*p*H 7.0), 0.01 *M* Versene, and 0.01 *M* cysteine, and mitochondria isolated by differential centrifugation.

Two possible explanations of this effect should be considered: (1) inhibition of the oxidase by carbon monoxide activates an alternative, nonphosphorylating respiratory system, which may be of the type previously described as the "cytochrome b-oxidase pathway" of plant mitochondria.[5] Preliminary evidence suggests that inhibition of electron transfer between cytochromes b and c also decreases the P/O ratio. (2) A change in the steady-state oxidation levels of the respiratory chain components decreases the free energy available for phosphorylation without interfering markedly with electron transfer. If this is true, it suggests that there may be a direct connection between the oxidation state of the respiratory carriers and the coupling mechanism.[6] With intact plant tissues, it frequently has been reported[5] that cytochrome oxidase inhibitors have unusual effects of the type produced by classical uncoupling agents. The present findings suggest that these effects may in fact be due to an interference with respiratory chain phosphorylations.

(1) This work was supported by the National Science Foundation and was reported previously at the ACS meeting in Boston, April, 1959.

(2) A. L. Lehninger, in "Phosphorus Metabolism" Johns Hopkins Press, Baltimore, Md., 1951, Vol. I, p. 344.

(3) B. Chance and G. R. Williams, *J. Biol. Chem.*, **221**, 477 (1956).

(4) D. P. Hackett and D. W. Haas, *Plant Physiol.*, **33**, 27 (1958).

(5) D. P. Hackett, *Ann. Rev. Plant Physiol.*, **10**, 113 (1959).

(6) C. L. Wadkins and A. L. Lehninger, *J. Biol. Chem.*, **234**, 681 (1959).

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RECEIVED JULY 6, 1959

PRECURSORS OF NICOTINIC ACID IN *Escherichia Coli*[1]

Sir:

In 1954[2] Yanofsky reported that *Escherichia coli* synthesizes nicotinic acid by a method different from the tryptophane–hydroxyanthranilic acid pathway which is used by neurospora and animals. Recent work from this laboratory has confirmed Yanofsky's observations and has also yielded information on the precursors of nicotinic acid in *E. coli*.

Resting cells of *E. coli* B were able to synthesize nicotinic acid (or a bound form of the vitamin) when the compounds listed in Table I were included in the reaction mixture. Fumarate, malate, or oxalacetate could substitute for succinate. Glyceric acid, or dihydroxyacetone could substitute for glycerol. Pyruvate was ineffective in replacing either glycerol or succinate. Tryptophane was inactive in the system. The addition of glucose to the reaction mixture inhibited synthesis of nicotinic acid. The requirements for ribose and adenine suggest that the synthesized product is a nucleotide or a nucleoside of nicotinic acid or nicotinamide rather than the free vitamin.

In order to prove that the carbon chains of the suspected precursors were being incorporated into nicotinic acid, reaction mixtures were prepared which contained succinic acid-2,3-C^{14}, glycerol-

(1) This work was supported by a grant from the National Science Foundation.

(2) C. Yanofsky, *J. Bact.*, **68**, 577 (1954).