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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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).

TABLE I

SYNTHESIS OF NICOTINIC ACID BY RESTING CELLS OF *E. coli*

The reaction mixture contained per 2 ml.: 20 mg. of cells (dry weight); 125 μM. phosphate buffer, *p*H 6.0; 75 μM. NH_4Cl; 20 μM. D-ribose; 10 μM. adenine; and 20 μM. of each of the different substrates. Incubation was for 3.5 hours at 37° (aerobically). After hydrolysis by heating at 120° for 30 min. in 1 *N* H_2SO_4, the nicotinic acid content of each reaction mixture was determined by microbiological assay with *Lactobacillus arabinosus* 17-5.[3]

Substrate added	Nicotinic acid content, mμg./mg. cells
None	222
None, acidified at 0 time	240
Succinate	362
Glycerol	396
Tryptophane	261
Succinate + glycerol	464
Succinate + glycerol (omit adenine)	231
Succinate + glycerol (omit ribose)	216
Succinate + glycerol (omit adenine and ribose	216

1,3-C^{14} and pyruvic acid-2-C^{14}. After incubation and hydrolysis with sulfuric acid an aliquot was removed from each vessel to determine the amount of nicotinic acid synthesized. Twenty mg. of carrier nicotinic acid then was added to the remainder of each reaction mixture. The nicotinic acid was isolated by passage through Dowex-50 and IRA-400 ion exchange resins and finally crystallized, as the picrate, and recrystallized to a constant melting point (210–215°) and constant specific radioactivity. Radioactive succinate and radioactive glycerol were both incorporated into nicotinic acid (Table II). The incorporation of C^{14}-

TABLE II

INCORPORATION OF C^{14} COMPOUNDS INTO NICOTINIC ACID

The reaction mixture and incubation conditions were as given in Table I

Substrate added	Specific activity of picrate of nicotinic acid c.p.m./μM.	Moles substrate incorporated per mole nicotinic acid synthesized
Glycerol-1,3-C^{14}	76	2.0
Succinate-2,3-C^{14} + pyruvate	44	1.0
Pyruvate-2-C^{14} + succinate	2	0
Glycerol-1,3-C^{14} + succinate + pyruvate	23	0.5

pyruvate was negligible. When C^{14}-glycerol was used as the only precursor, two moles of glycerol was incorporated per mole of nicotinic acid synthesized. This suggests that glycerol is capable of supplying all of the carbon atoms of nicotinic acid.

(3) E. E. Snell and L. D. Wright, *J. Biol. Chem.*, **139**, 675 (1941).

(4) Supported by a Karl T. Compton fellowship from the Nutrition Foundation (1956–1958) and the Banco de México, S.A.

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STABLE MERCUROUS COMPLEXES

Sir:

When a complexing ligand is added to a mercurous solution, the usual reaction that occurs is disproportionation of the mercurous ion to give elementary mercury and a complexed mercuric ion. In fact, several well-known textbooks assert that there are no known complexes of mercurous ion. It has been suggested recently that Hg_2^{++} does exhibit weak ion pair association with ClO_4^- and NO_3^- with formation constants of 0.9 M^{-1} and 2.5 M^{-1}.[1] Some time ago, Stromeyer[2] reported that when sodium pyrophosphate solution is added to a mercurous solution, a white precipitate forms and then redissolves in excess of the reagent, which suggests the formation of a strong, stable complex.

We have now found that the mercurous ion forms stable soluble complexes with pyrophosphate, oxalate, succinate, and tripolyphosphate. The results with pyrophosphate will be described in detail. There is no doubt that mercury of oxidation state (I) is still present in the clear solution of mercurous nitrate in excess sodium pyrophosphate, since Hg_2Cl_2 can be precipitated by addition of NaCl solution. The mercurous solutions are also formed by reaction of Hg(II) pyrophosphate complexes with elementary mercury, so the complex is stable to disproportionation. A potentiometric study indicates that the predominant species are $Hg_2Py_2^{-6}$, and $Hg_2Py(OH)^{-3}$ (where $Py = P_2O_7^{-4}$).

The potential of a Hg,Hg(I) electrode in solutions at *p*H's 7–10, $\mu = 0.75$ *M* ($NaNO_3$), (Hg_2(I)) = 5×10^{-5} to 2×10^{-3} *F*, (Na_4Py) = 0.004–0.05 *F*, *T* = 27.5°, can be interpreted in terms of the two equilibria (*M* = mole liter^{-1})

$$Hg_2^{++} + 2\ Py^{-4} \rightleftharpoons (Hg_2Py_2)^{-6} \qquad K_2 = 2.4\ (\pm 0.6) \times 10^{12}\ M^{-2}$$

$$Hg_2^{++} + OH^- + Py^{-4} \rightleftharpoons (Hg_2Py(OH))^{-3} \qquad K_1 = 1.3\ (\pm 0.2) \times 10^{16}\ M^{-2}$$

The predicted equation for the potential considering the above equilibria, is E(mv.) = E^0 + 29.7 [log (ΣHg_2^{++}) − log (1 + K_1(OH)(Py) + K_2(Py)^2)], with E^0 *vs.* SCE = 539 mv. Representative data are displayed in Table I. Roughly speaking, $(Hg_2Py_2)^{-6}$ is the main species at Σ(Py) = 0.02 *F* for *p*H's between 7 and 8.5; Hg_2Py-

TABLE I

POTENTIAL MEASUREMENTS

*p*H	(ΣHg_2^{++}) $\times 10^5$ *F*	(ΣPy) $\times 10^{22}$ *F*	*E* (*vs.* S.C.E.) mv.	*E* (calcd.)
9.10	7.55	0.484	151.34	153.5
	6.71	2.571	124.10	126.0
	6.25	3.723	118.70	119.2
8.39	7.57	0.484	177.29	174.7
	7.01	1.877	150.10	151.3
	3.83	4.90	121.72	122.5
	5.71	4.90	127.16	127.1
	7.51	4.90	131.22	130.6

[a] We measure the effective *pK* of HPy^{-3} in this medium as 8.00; *cf.* Lambert and Watters.[3]

(1) S. Hietanen and L. G. Sillén, *Arkiv Kemi*, **10**, 103 (1956).

(2) F. Stromeyer, *Schweigger's Journal*, **58**, 130 (1830); as reported in J. W. Mellor, "A Comprehensive Treatise on Inorganic and Theoretical Chemistry," Longmans, Green and Company, London, 1923; Vol. IV, p. 1003.

(3) S. M. Lambert and J. I. Watters, THIS JOURNAL, **79**, 4262 (1957).