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.

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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-

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