C, 47.2; H, 4.0; V, 20.3; Cl, 28.0). Bis-cyclopentadienylvanadium(IV) dichloride is soluble in chloroform, ethyl acetate and alcohol; it decomposes on heating above 250°. In water it forms a green unstable solution which gives the precipitation reactions typical of bis-cyclopentadienyl metal ions. A dark green picrate (*Anal.* Calcd. for $C_{22}H_{14}N_6O_{14}V$: N, 12.8; V, 7.6. Found: N, 12.8; V, 7.7) has been precipitated from this solution. Bis-cyclopentadienylvanadium(IV) dichloride is paramagnetic with one unpaired electron ($\chi_{mol}^{23°C} = +1600 \times 10^{-6}$ c.g.s.u., corrected for diamagnetic contribution; $\mu_{eff} = 1.95$ B.M.).

The infrared absorption spectra of bis-cyclopentadienylnickel(II) and the bis-cyclopentadienyl dibromides of titanium, zirconium and vanadium are similar to those of ferrocene[1a] and ruthenocene.[4]

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THE ACCUMULATION OF ACETYLMETHYLCARBINOL (3-HYDROXY-2-BUTANONE) BY ACETATE-REQUIRING MUTANTS OF *NEUROSPORA CRASSA*[1]

Sir:

The biological oxidation of pyruvate to acetate occurs in a number of steps, the first of which apparently includes decarboxylation of the pyruvate with the formation of a two-carbon–enzyme complex at the oxidation level of acetaldehyde.[2] This C_2-enzyme complex is then oxidized, as a complex, after which the oxidation product can be hydrolyzed to give acetic acid. Evidence for the first step has been obtained by Schweet, *et al.*,[3] who showed that enzyme preparations forming acetate can also form acetylmethylcarbinol (AMC-3-hydroxy-2-butanone) presumably by reaction of the C_2-enzyme complex at the acetaldehyde oxidation stage.

Additional evidence relating to the initial stages of pyruvate oxidation has been obtained using a series of mutants of *Neurospora crassa* which require acetate for growth. These mutants are deficient in their ability to oxidize pyruvic acid.[4] The acetate-requiring strains 50–6[4], S34, S48, S48+sp, S210[5] accumulate a volatile substance giving a positive Voges–Proskauer reaction on standing. This substance has been identified as AMC on the basis of the following criteria: (a) distillates of media in which acetate mutants have grown give a positive Voges–Proskauer reaction; (b) no reaction is observed on treating distillates of media with hydroxylamine and nickelous chloride but a heavy red precipitate characteristic of nickel dimethylglyoxime is obtained after oxidation with ferric chloride[6] and redistillation; (c) the 2,4-dinitrophenylhydrazones of the substances, obtained by the method of Green, *et al.*,[7] from distillates of the culture media of S48 and S48+sp. (grown six days in 20 ml. of minimal medium[8] containing 20 mg. of acetic acid as the sodium salt), gave decomposition points similar to that reported for the dinitrophenylhydrazone of diacetyl (317°, reported 315°) and there was no depression of the decomposition point on mixing the derivative from S48+sp with dinitrophenylhydrazone prepared from an authentic sample of diacetyl.

Neurospora metabolizes pyruvate via a carboxylase as well as by an oxidative system.[4] This carboxylase reaction is involved in ethanol production. That the carboxylase system is not involved in the production of AMC by acetate mutants is indicated by Table I. Acetate requiring strains with both high and low carboxylase activities accumulate large amounts of AMC. Acetate independent strains do not accumulate significant amounts of AMC regardless of their alcohol production or carboxylase activity.

TABLE I

ACCUMULATION OF ACETYLMETHYLCARBINOL BY MUTANTS OF *Neurospora*

Strains grown 4 days on minimal medium[8] containing 20 mg. of acetic acid as the sodium salt. AMC was determined by the method of Westerfeld, *et al.*,[9] alcohol was determined by the method of Friedmann and Klass.[10] Carboxylase was assayed as the μl. of CO_2 evolved in 10 minutes at *p*H 5.3 from 9×10^{-2} *M* pyruvate at 37°. The extract used for carboxylase assay was the supernatant fraction of a mycelial homogenate.

	Strain			
	Acetate-requiring		Acetate-independent	
	S48	S48+sp	8a	50-8
Dry weight, mg.	38.3	28.3	71.2	87.6
Acetylmethylcarbinol accumulated g./mg., dry wt.	175	450	0.9	0.2
Ethanol accumulated mg./mg. dry wt.	0.64	0.07	1.25	0.32
Carboxylase activity μl. CO_2/10 min./mg. N	156	16	285	101

The acetate requiring mutants of *Neurospora* are apparently blocked in pyruvic acid oxidation after the formation of a C_2-enzyme complex at the acetaldehyde oxidation stage. As a result of the block preventing oxidative metabolism of the complex, the complex reacts with a C_2 compound at the acetaldehyde oxidation stage or with pyruvate to give AMC. This mechanism is consistent with, and supports, present ideas of the initial stages of pyruvate oxidation. Since at least three genetically different acetate requiring mutants accumulate AMC further studies of the biochemical differences among these mutants can be expected to help elucidate the details of pyruvate oxidation.

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