THE OXIDATION OF ACETONE BY A SOIL DIPHTHEROID

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In recent months a series of papers have appeared, namely by Price and Rittenberg (1950), Plaut and Lardy (1950), and Sakami (1950), which indicate that acetone is utilized by mammalian tissue. Several metabolic pathways have been proposed for this oxidation. Plaut and Lardy (1950) concluded that acetone was directly incorporated into acetoacetate by a mechanism involving CO\textsubscript{2} fixation. Price and Rittenberg (1950) demonstrated the conversion of acetone to an acetylating agent by the mammalian organism.

Sakami (1950) reported that when acetone labeled in the methyl groups with C\textsuperscript{14} was administered to rats, the labeled carbon appeared in the beta carbon of serine and the methyl carbon of choline and methionine. Similar labeling of these compounds occurred when C\textsuperscript{14} formate was administered to rats. This suggested to Sakami that the synthesis of labile methyl groups might occur over a pathway involving formate or a similar compound. His data are consistent with the hypothesis that there is a primary oxidative attack on acetone followed by a 2 and 1 carbon cleavage in which acetate and formate or some closely related compounds are formed.

Rudney (1950) has observed that the administration of C-1 labeled propylene glycol to rats resulted in the appearance of C\textsuperscript{14} in the same compounds and in the same positions as when methyl labeled acetone was utilized.

It appeared that the problem of acetone oxidation might be more readily attacked using microorganisms. In addition, it was conceived that the oxidation of acetone might proceed over a pathway involving acetol (CH\textsubscript{3}COCH\textsubscript{2}OH) as an intermediate. Acetol then might undergo cleavage to yield acetaldehyde and a 1 carbon unit which could serve as a precursor of methyl groups. The following reactions illustrate the postulated pathway for acetone oxidation.

\begin{equation}
\text{CH}_3\text{COCH}_2 + \text{O}_2 \rightarrow \text{CH}_3\text{COCH}_2\text{OH} \rightarrow \text{CH}_3\text{CHO} + \text{C}_1 \text{unit}
\end{equation}

A survey of the microbiological literature revealed the almost complete absence of studies dealing with the utilization of acetone by microorganisms. Supniewski (1923) reported that \textit{Bacillus pyocyaneus} would grow in a medium containing 0.23 per cent acetone and that it produced acetic and formic acids as end products of growth. Goepfert (1941), in the course of studies on the dehydrogenations carried out by \textit{Fusaria}, was able to trap formaldehyde when cultures were incubated in an acetone medium and acetol and formaldehyde when cultures were grown on propylene glycol. Since the initiation of the studies

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to be described here, Siegel (1950) has reported data which implicate acetoacetate as an intermediate in acetone utilization by the photosynthetic bacterium, *Rhodopseudomonas gelatinosa*.

*Isolation of an acetone oxidizing organism.* On the basis of the report by Supniewski (1923), we tested several stock strains of *Pseudomonas aeruginosa* for their ability to utilize acetone, with negative results. After trying unsuccessfully to isolate an acetone oxidizing organism from soil using an enrichment medium consisting solely of inorganic salts and acetone, the following enrichment medium was prepared: per 100 ml 0.025 M Sörensen's phosphate buffer, pH 7.0, 200 mg acetone, 10 mg yeast extract, 20 mg MgSO₄·7H₂O, 4 mg MnSO₄·4H₂O, 1 mg NaCl, 1 mg FeSO₄·7H₂O.

The inoculum for the above medium consisted of soil taken from the shoreline of a local pool. The inoculated medium was incubated at 30° C in an atmosphere of oxygen. Subcultures were made when growth had occurred in the enrichment culture, about 2 to 3 days. At the end of 10 days a sample of the mixed culture was tested manometrically for its ability to oxidize acetone, with positive results. At this point the mixed culture was streaked on nutrient agar, and a diphtheroid capable of oxidizing acetone was isolated. We have as yet been unable to identify the organism beyond assigning it to the genus *Corynebacterium*. It is interesting to note that although the organism is a typical diphtheroid on nutrient agar, it exhibits the morphology of a coccus when grown in the liquid acetone medium described above.

**MANOMETRIC EXPERIMENTS**

*Methods.* The cells were grown at 30° C with constant shaking. The medium used for growth consisted of the basal medium described above except that the different carbon sources, whose effects were being studied, were substituted for acetone. The inoculum consisted of cells grown at 30° C on the acetone medium. After 2 to 4 days the cells were harvested, washed with distilled water, and resuspended in 0.1 M Sörensen's phosphate buffer (pH 7.0) to yield a suspension containing 200 mg wet weight of cells per ml. One-half ml of this suspension was added to each Warburg vessel together with distilled water and substrate to yield a final volume of 2 ml. Standard manometric procedures were used. Acetone was determined by the method of Greenberg and Lester (1944). Acetol was synthesized by the method of Levene and Walti (1943). Acetol was determined by periodate oxidation (Jackson, 1944).

**RESULTS**

*Effect of yeast extract concentration.* The ability of cell suspensions of this organism to oxidize acetone is apparently an adaptive process. Cells obtained from cultures of the enrichment medium containing more than 0.01 per cent yeast extract were incapable of oxidizing acetone.

*Adaptation experiments.* Experiments were designed to determine the effect of growth on different carbon sources, on the ability of this organism to oxidize acetone, and the postulated intermediates in its oxidation, namely acetol and
acetaldehyde. Since Rudney (1950) had conceived of propylene glycol as an intermediate in acetone oxidation by mammalian tissue, it was included in these experiments.
Resting cells obtained from the growth medium containing glucose as the carbon source were able to oxidize glucose, but unable to oxidize acetone, acetaldehyde, acetol, or propylene glycol.

Growth on acetone yielded cells that were able to oxidize acetone, acetol, and acetaldehyde, but not propylene glycol (figure 1).

Cells obtained from growth in a propylene glycol medium were capable of oxidizing propylene glycol, acetol, and acetaldehyde, but not acetone, as shown in figure 2.

Cells grown under all of the above conditions were able to oxidize glucose and pyruvate (not illustrated). It would appear that cells grown on either acetone or propylene glycol are adapted to the oxidation of acetol, acetaldehyde, and the carbon source used for growth, but not to the oxidation of the other compound.

Isotopic experiments. To further verify the suggested role of acetol as an intermediate in acetone oxidation, it was decided to investigate the ability of this organism to incorporate C\textsuperscript{14} from isotopically labeled acetone into unlabeled

| TABLE 1

Incorporation of C\textsuperscript{14} from carbonyl labeled acetone into acetol by the soil diphtheroid |
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<td>EXPERIMENT NO.</td>
<td>CH\textsubscript{3}CO CH\textsubscript{2}OH</td>
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<tr>
<td></td>
<td>cpm per mm</td>
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<tr>
<td>1</td>
<td>35,000</td>
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90 \( \mu \text{M} \) carbonyl labeled acetone, \( 3.9 \times 10^4 \) cpm, incubated with 1 g of cells and 300 \( \mu \text{M} \) acetol for 20 to 30 min at 30 C.

carrier acetol. A washed suspension of acetone grown cells was permitted to oxidize acetone, labeled in the carbonyl position with C\textsuperscript{14}, in the presence of unlabeled acetol as carrier. After a short period of time (20 to 30 minutes) the oxidation was stopped by the addition of acid. After removal of the cells by centrifugation, the neutralized supernatant was taken to dryness under vacuum, the distillate being trapped in liquid nitrogen. Control experiments showed that added acetol and acetone could be quantitatively recovered by this method.

Since the acetol and acetone could not be separated directly, the distillate was treated for 30 minutes with sodium metaperiodate which oxidized the acetol to acetate and formaldehyde. The acetate resulting from this oxidation contained the carbonyl carbon of acetol, while the formaldehyde contained the carbinol carbon of the acetol. Acetone is not oxidized by metaperiodate under the conditions used. The excess metaperiodate was reduced to iodate by adding several drops of ethylene glycol. The resulting mixture of acetone, acetate, and formaldehyde was separated by steam distillation.

Using the above procedure, 75 per cent of the carbonyl labeled acetone and 50 to 60 per cent of the added carrier acetol were reisolated at the end of the experiment. The acetate was converted to \( \text{CO}_2 \) by persulfate oxidation (Calvin
et al., 1949) and counted as BaCO₃, appropriate correction being made for self absorption. The formaldehyde was isolated as the dimedon derivative and counted as such, according to the method of Berg (1951). An end window Geiger-Mueller counter was used.

Table 1 illustrates the results of two such experiments. It can be seen that radioactivity was incorporated into the acetol. In addition, the activity was associated with the carbonyl position since all of the activity was found in the acetic acid and none in the formaldehyde resulting from the periodate oxidation of the carrier acetol. The formaldehyde fraction not only contained formaldehyde resulting from the oxidation of acetol by metaperiodate, but also formaldehyde from the oxidation of the ethylene glycol used to remove the excess reagent. Since there was no radioactivity in the formaldehyde fraction, it is reasonable to assume that the ethylene glycol did not materially contribute to any error of measurement of radioactivity of this fraction. The above distribution of isotope is to be expected, if acetol were an intermediate in acetone oxidation and if it arose by a direct oxidation of acetone to acetol.

DISCUSSION

The experiments reported above strongly suggest that acetol is an intermediate in the oxidation of acetone by this organism. It would appear that the acetol then undergoes a 2 and 1 carbon cleavage in which acetaldehyde or some closely related compound is formed. This conclusion is suggested by the positive correlation between the ability of the cells to oxidize acetone, acetol, and acetaldehyde. The evidence, therefore, supports the hypothesis presented in equation I as to the pathway of acetone oxidation.

It would also appear that propylene glycol is neither a precursor of acetone nor an intermediate in its oxidation. Instead both compounds seem to be oxidized via a common pathway involving acetol and acetaldehyde as follows:

\[
\begin{align*}
    &\text{CH}_3\text{COCH}_2\text{OH} \\
\rightarrow &\text{CH}_3\text{CHOHCH}_2\text{OH} \\
\downarrow &\text{CH}_3\text{CHO} + \text{C}_1 \\
\end{align*}
\]

The pathway for propylene glycol oxidation appears to be similar to the pathway occurring in Fusarium tini-bolley as reported by Goepfert (1941). The presence of a similar pathway in mammalian tissue would account for the observations of Rudney (1950) with C-1 labeled propylene glycol (cited above). The occurrence of such a pathway in mammalian tissue remains to be demonstrated.

SUMMARY

A diphtheroid has been isolated from soil that is capable of oxidizing acetone. The ability to oxidize acetone is adaptive, requiring growth of the organism in its presence.
Adaptive experiments suggest that acetol and acetaldehyde are intermediates in the oxidation of acetone.

Propylene glycol is neither a precursor of acetone nor an intermediate in its oxidation. Instead, propylene glycol appears to be oxidized via the same pathway as acetone, with acetol and acetaldehyde serving as intermediates.

The role of acetol as an intermediate in acetone oxidation has also been demonstrated by isotopic experiments.

REFERENCES