THE EFFECT OF AMINO GUANIDINE ON THE OXIDATION OF
FORMALDEHYDE BY RAT LIVER

BY FREDERICK BERNHEIM

(From the Department of Physiology and Pharmacology, Duke University
School of Medicine, Durham, North Carolina)

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Formaldehyde was shown to be a normal metabolite when Handler,
Bernheim, and Klein (1) found sarcosine oxidase which catalyzes the
oxidative demethylation of sarcosine to glycine and formaldehyde. Sakami
(2), Welsh and Sakami (3), and Stekol et al. (4) have recently presented
evidence that formate may provide the animal with necessary methyl
groups. It was therefore of interest to investigate the oxidation of formal-
dehyde to formic acid in vitro in animal tissue. It apparently has been
assumed that xanthine oxidase or aldehyde oxidase is responsible for its
metabolism.

EXPERIMENTAL

Rat liver was ground in a mortar with 0.05 M Na-K-phosphate buffer,
1.0 ml. per gm., and strained through muslin. 0.5 ml. of the resulting
suspension was used in each Warburg vessel with a fluid volume of 2.0 ml.
Washed preparations were made by centrifuging the suspension after
diluting with water to 50 ml., discarding the suspension, and washing again
with buffer and water.

When 0.1 mg. of formaldehyde was added to the unwashed suspension,
the oxygen uptake was inhibited for 10 to 20 minutes. The inhibition then
disappeared and the formaldehyde was apparently oxidized fairly rapidly
to formate, which was then oxidized more slowly to carbon dioxide. When
0.2 mg. of formaldehyde was added, the same sequence occurred except
that the inhibition was greater and more prolonged. With larger amounts
the inhibition was not overcome and there was no apparent oxidation.
When washed preparations were used, formaldehyde was not oxidized.

These facts suggested that formaldehyde first inhibited some oxidative
enzymes from which it slowly dissociated to be oxidized to formate, or that
it combined eventually with amino groups of soluble compounds and was
oxidized when still combined. On the assumption that the latter mechan-
ism was more likely, formaldehyde was mixed with a number of amino
compounds before it was added to the liver suspension. These included
amino acids, amines, ammonium salts, urea, hydrazine, phenylhydrazine,
guanidine, and aminoguanidine. All were without effect except the last.
When aminoguanidine and formaldehyde were mixed and then added to the liver suspension, a rapid oxygen uptake occurred. Aminoguanidine itself was without effect. The uptake was proportional to the concentration of formaldehyde and independent within limits of the concentration of aminoguanidine. The latter was not acting as a catalyst. As shown in Fig. 1, the uptake required approximately equimolar amounts of both substances to get maximum effects. If too little aminoguanidine was present, some formaldehyde either remained uncombined or reacted with the other amino groups in the molecule. If too much was present, polymerization probably occurred, for both the rate and amount of oxygen uptake decreased. It thus appears that formaldehyde is oxidized when combined with aminoguanidine. In confirmation of this it is possible to mix equimolar amounts of the two, and evaporate to dryness; the resulting product, which is amorphous, was rapidly oxidized when added to liver with an uptake proportional to the amount added. If less than an equimolar amount of formaldehyde was used, the resulting product was oxidized slowly or not at all.

Fig. 1. The oxidation of two concentrations of formaldehyde with and without different concentrations of aminoguanidine sulfate by rat liver suspension at pH 7.8 and 37°. The control oxygen uptake of the liver has been subtracted in each case. The theoretical uptake for the oxidation of 0.08 mg. of formaldehyde to carbon dioxide is 60 cmm. of oxygen.
The amount of oxygen taken up shows that formaldehyde was oxidized to carbon dioxide and water. Formate, added to liver suspensions, was oxidized and the rate was not affected by aminoguanidine. If the suspension was washed, neither formaldehyde nor formate was any longer oxidized and the formaldehyde-aminoguanidine complex was oxidized to the formate stage. This is shown in Fig. 2. The inability of washed liver suspension to oxidize the formaldehyde may mean that a substance in liver with which formaldehyde can combine had been washed away or that an enzyme which may oxidize it in the free state had been inactivated. In neither washed nor unwashed suspensions did aminoguanidine catalyze the oxidation of acetaldehyde or other straight chain and aromatic aldehydes. When formaldehyde was released from sarcosine by the action of the sarcosine oxidase, aminoguanidine, added to the suspension, combined with it and increased the oxygen uptake. Acetaldehyde produced by the action of the alcohol oxidase on ethyl alcohol is not affected under the same conditions.

Two experiments show that the xanthine oxidase is not responsible for...
the oxidation of the aminoguanidine-formaldehyde complex. First, the milk enzyme which oxidizes formaldehyde does not oxidize the complex. Incidentally, this is a further indication that the complex does not dissociate to release formaldehyde. Secondly, quinimine, which inhibits the xanthine oxidase of rat liver (5), has no effect under the same conditions on the oxidation of the complex. This is shown in Fig. 3. The identity of the enzyme responsible for the oxidation has not been determined. Since it is not inhibited by $1 \times 10^{-2} \text{M}$ cyanide, it cannot be either the alcohol or choline oxidases. It is not inhibited by relatively large amounts of guanidine, urea, or hydrazine, nor by amino acids. It is present in rat kidney in a lesser amount and absent from brain. It is absent from guinea pig and hamster liver and kidney. Its pH optimum is approximately 8.0; it is completely inactive at pH 6.0. Its activity is destroyed by heating for 2 minutes in a boiling water bath.

**DISCUSSION**

It is possible to write the reaction between aminoguanidine and formaldehyde as follows:

$$\text{NHNH}_2 + \text{NH}_2\text{CH}_2\text{OH} \rightarrow \text{C}=\text{NH} + \text{CH}_2\text{O}$$

The compound resembles no known metabolite and it is not possible to attribute its oxidation to any recognized enzyme. On the basis of these results, however, it can be assumed that a similar reaction may take place between formaldehyde and a normal component of tissue.

**SUMMARY**

1. Formaldehyde is rapidly oxidized by rat liver if it is mixed in approximately equimolar amounts with aminoguanidine. Guanidine, amines, amino acids, hydrazine, and urea are without effect.

2. The properties of the enzyme catalyzing the oxidation are described.

**BIBLIOGRAPHY**

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Frederick Bernheim


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