STUDIES ON THE METABOLISM OF METHANOL AND FORMALDEHYDE IN THE ANIMAL ORGANISM

BY

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INTRODUCTION

Methanol (CH₃OH) is the lowest member of the series of primary saturated aliphatic alcohols. Its biological behaviour is markedly different from that of the next homologue, ethanol. Although the acute toxicity of methanol is apparently lower than that of ethanol, methanol has a specific, highly toxic late effect, i.e., its subacute and chronic toxicities are high. Very typical for methanol poisoning in man is the transitory or permanent blindness, amblyopia, which already may follow the ingestion of small amounts of methanol. Since methanol poisonings are rather usual especially during times of war and shortages, considerable attention has been devoted to methanol especially in toxicological and clinical literature. The significance of methanol, and especially that of its metabolites formaldehyde and formic acid, does not, however, seem to be confined to the field of toxicology. Certain nutrients, as, for instance, fruits, contain much pectin from which methanol is liberated in the organism. The tremendous advance during recent years in our knowledge of the metabolism of the so called one-carbon compounds has demonstrated that formaldehyde and formic acid or their derivatives are of a great significance in normal intermediary metabolism. The organism is capable of also using methanol for the formation of many important compounds.

In the following chapter a general survey is given of the literature concerning the metabolism of methanol and formaldehyde in the animal organism. Clinical and pathological aspects have not been discussed and the reader is referred in this respect to the monographs and papers of Røe (1943, 1946, 1955) and Orthner (1955). More detailed surveys of the rapidly expanding literature on the metabolism of one-carbon compounds can be found
which enables it to prevent the adsorption of methanol to the oxidative enzymes and consequently its conversion to formic acid. On the basis of these observations he also advocates the use of ethanol in addition to bicarbonate in the therapy of methanol poisoning.

Orthner (1950) takes an entirely opposite standpoint in regard to the effect of ethanol. He states that ethanol taken simultaneously with methanol dilates the blood vessels and raises the permeability, thus increasing the special toxic effects of the metabolic products of methanol.

In white mice the median lethal dose (LD₅₀) for a single intraperitoneal injection of methanol is significantly decreased by ethanol (Gilger, Potts and Johnson 1952). Similar results have also been obtained in experiments with guinea pigs (Moeschlin and Garson 1955). However, these experiments have dealt mainly with the acute toxicity of methanol, which is due to its narcotic effects and is much smaller than that of ethanol.

According to von Fellenberg (1917), the low urinary excretion of methanol found after pectin-rich food is considerably increased when ethanol is also consumed. He suggested that the oxidation of methanol becomes more difficult when the organism must simultaneously oxidize large amounts of ethanol.

Asser (1914) found in experiments on dogs and rabbits that when he gave ethanol simultaneously with methanol the increased excretion of formic acid in the urine decreased appreciably. Acetone and amyl alcohol had a similar effect. As the most appropriate explanation he considered the possible increase in the oxidation of the formic acid due to an increased permeability of the cells. This decrease in the urinary excretion of formic acid after the simultaneous intake of ethanol and methanol has since been confirmed in experiments with dogs and rabbits (Bastrup 1947) as well as in man (Kendal and Ramanathan 1953). In the latter it was found that when the dosage of methanol was 4 ml, about 10 ml of ethanol per hour were required to suppress totally the increased urinary excretion of formic acid.

Human experiments were also conducted by Leaf and Zatman (1952). In these experiments ethanol was taken at various times during the elimination of a fixed dose, 4 ml, of methanol. A single dose of 15 ml ethanol taken simultaneously caused a marked elevation in the peak concentration of methanol attained in the urine. When ethanol was taken after methanol it effectively arrested the decline in the urinary methanol concentration. After about two hours the decline occurred at the original rate. In all of their experiments it was seen that when ethanol was administered during the elimination of methanol, the decline in the methanol concentration in the urine was very slow. The reason for this effect of ethanol the authors consider to be an inhibition of the oxidation of methanol by alcohol dehydrogenase.

The oxidation of isotope-labelled methanol to carbon dioxide in rats in vivo is strongly inhibited by the simultaneous administration of ethanol (Bartlett 1950 b). When a sufficient amount of ethanol was given the oxidation of methanol ceased totally, and after the apparent oxidation of ethanol that of methanol continued at the same rate as before the administration of ethanol. The inhibitory action of ethanol on the metabolism of methanol was apparent also in the experiments of Agner and Belfrage (1946), in which they administered simultaneously methanol and ethanol to two rabbits. The elimination of methanol from blood was distinctly retarded by ethanol.

We have very little knowledge of the effects of ethanol on the metabolism of methanol in in vitro systems. Zatman (1946) mentioned in a brief note the results of experiments on the effect of ethanol on the oxidation of methanol by the crude alcohol dehydrogenase from horse liver prepared according to Luwax-Mann (1938). This preparation was found to oxidize methanol at about one-ninth of the rate for the oxidation of ethanol. When mixtures of methanol and ethanol were used as substrate the oxidation of methanol was inhibited by ethanol, as judged from the decreased amount of formaldehyde formed. When the alcohols were present in equimolar amounts no formaldehyde was formed and the inhibition was still distinct when the molar ratio of ethanol and methanol was only 1:16.

Added ethanol depressed the oxidation of isotope-labelled methanol by rat liver slices as a linear function of the logarithm of the ethanol concentration (Bartlett 1950 b). In these experiments a 72 per cent inhibition of the carbon dioxide formation from methanol was obtained when both ethanol and methanol were present in a concentration of 0.04 M.

Pathogenesis of Methanol Poisoning

Since the end of the past century, when the first cases of human methanol poisoning were described, the cause of the specific toxicity of methanol has been much debated. Although many different hypotheses, some purely speculative and others based on clinical or experimental observations, have been presented, we do not as yet exactly know how the specific toxic action of methanol is brought about.

It was earlier a rather generally accepted opinion that methanol itself is not very toxic and that the toxicity depends on various impurities in the methanol (fusel oil) (Stadelmann and Magnus-Levy 1912, Igersheimer and Verzar 1913, Rostedt 1922, Hamalainen and Terakskeli 1928, and others). This theory, however, had to be abandoned since it was repeatedly shown that also chemically pure, synthetically produced methanol was the cause of numerous poisonings (e.g., Reif 1923, Alder, Buschke and Gordonoff 1938). The acute toxicity of methanol is lower than that of ethanol, and the so-called Richardson's law that the toxicity of alcohols increases in the homologous series in the ratio 1:3:3'... was shown to be valid also in the case of methanol and ethanol (Fühner 1955,
They have been suggested as a factor in the development of the acidosis. The formation of formaldehyde as a normal metabolite in the animal organism was denied for a long time and only a toxicological role was given to it. During the past few years, however, when our knowledge of the metabolism of one-carbon compounds has greatly advanced, formaldehyde has also gained new significance. It has now been shown to be a possible intermediate in many important biological reactions.

The reactions of formic acid in the intermediary metabolism can be divided into four groups, i.e., reactions where formaldehyde is formed, directly oxidative reactions, various condensation reactions, and reactions which link it to the metabolism of the one-carbon group.

Formation of Formaldehyde

The formation of formaldehyde from methanol has been discussed in the earlier chapter. However, formaldehyde may also be formed from other, in some cases more physiological compounds than methanol.

When various N-methylated compounds are demethylated by animal tissue preparations, it has been found that formaldehyde is formed from the methyl group. Handler, Bernheim and Klein (1941) found that broken cell preparations of rabbit, rat and guinea pig liver are able to oxidatively demethylate sarcosine to glycine. They obtained strongly positive qualitative reactions for formaldehyde in the solution of oxidized sarcosine.

Mackenzie and Du Vigneaud (1949) and Mackenzie (1950) studied further the biological oxidation of the methyl group of sarcosine in rat liver homogenates. They obtained radioactive formaldehyde as an oxidation product of sarcosine labelled in the methyl group with C\textsuperscript{14}. Formaldehyde was also isolated and identified by them in the form of dimedone derivative.

All the methyl groups which are known to be oxidized to formate and carbon dioxide do not apparently produce formaldehyde with the same facility during this reaction. Formaldehyde was obtained from sarcosine, dimethylaminoethanol, dimethylglycine and methanol incubated with rat liver preparations, but not from methionine, betaine, choline, glycine, serine, monomethylaminoethanol or aminoethanol by Mackenzie, Johnston and Prisell (1953). The degradation of serine to glycine and formaldehyde, on the other hand, has been demonstrated in various liver preparations by Vilenkina (1949, 1952), Blakley (1954 a, b) and Alexander and Greenberg (1955). Siekevitz and Greenberg (1950) obtained labelled formaldehyde from the methyl groups of methionine and choline and from the α-carbon of glycine and β-carbon of serine after incubation
Utilization of Methanol and Accumulation of Formaldehyde in Homogenates of Other Tissues

Since most of the experiments recorded in this study were made with liver tissue homogenates, comparative studies on the utilization of methanol and the accumulation of formaldehyde in other tissues were made.

The results of a representative series of experiments on homogenates made from various guinea pig tissues are recorded in Table 6. Pooled tissues from three animals were used for the preparation of the homogenates. Three different incubations were simultaneously carried out with each tissue, one incubation being done without added substrate as a control for possible endogenous variations during the incubation. Similar results were obtained in corresponding experiments with the same tissues of other guinea pigs. No significant differences were noted in the relative activities of the tissues of guinea pig and rat, but the absolute values tended to be somewhat higher in rat tissues; however, there were considerable variations in these results. In Table 7 are presented the results of a series using rat tissues.

Liver tissue was found to be superior to other tissues in its ability to utilize methanol. The accumulation of formaldehyde was likewise highest in the liver homogenates.

However, other tissues can also utilize methanol but at a lower rate. Kidney cortex homogenates can utilize methanol, although at a rate which is about one-third of that of liver tissue homogenates. Despite the considerable utilization of methanol, only small amounts of formaldehyde were accumulated in most kidney cortex homogenates, which seems to indicate highly active utilization of formaldehyde in the kidney tissue. Spleen tissue was found to be very active in the utilization of methanol in some preparations. In some animals this utilization was greater than in kidney homogenates, but in others there was only a negligible utilization of methanol. Some methanol was also utilized in homogenates of testis tissue.

The ability of lung tissue homogenates to utilize methanol and the accumulation of formaldehyde varied very much in the different preparations, but some utilization was always present. This activity of lung tissue, which is usually considered rather inert in metabolic reactions, is remarkable. In most preparations the accumulation of formaldehyde in comparison to the amount of methanol utilized was much greater in lung tissue homogenates than in liver and kidney tissue homogenates.

Although muscle tissue is generally regarded as very active metabolically, homogenates from skeletal muscle could not utilize methanol in demonstrable amounts. Heart muscle homogenates had a slight activity in utilizing methanol, and some formaldehyde was accumulated. Homogenates of brain tissue showed no activity.

Table 6

Utilization of methanol and accumulation of formaldehyde in homogenates of different guinea pig tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Methanol utilized</th>
<th>Formaldehyde accumulated</th>
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<tbody>
<tr>
<td>Liver</td>
<td>1550 µg</td>
<td>136 µg</td>
</tr>
<tr>
<td>Kidney</td>
<td>530 µg</td>
<td>20 µg</td>
</tr>
<tr>
<td>Spleen</td>
<td>460 µg</td>
<td>28 µg</td>
</tr>
<tr>
<td>Lung</td>
<td>365 µg</td>
<td>48 µg</td>
</tr>
<tr>
<td>Testis</td>
<td>185 µg</td>
<td>17 µg</td>
</tr>
<tr>
<td>Heart muscle</td>
<td>40 µg</td>
<td>5 µg</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>0 µg</td>
<td>1 µg</td>
</tr>
<tr>
<td>Brain</td>
<td>0 µg</td>
<td>0 µg</td>
</tr>
</tbody>
</table>

Table 7

Utilization of methanol and accumulation of formaldehyde in homogenates of different rat tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Methanol utilized</th>
<th>Formaldehyde accumulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>1890 µg</td>
<td>143 µg</td>
</tr>
<tr>
<td>Kidney</td>
<td>680 µg</td>
<td>47 µg</td>
</tr>
<tr>
<td>Spleen</td>
<td>310 µg</td>
<td>24 µg</td>
</tr>
<tr>
<td>Lung</td>
<td>235 µg</td>
<td>60 µg</td>
</tr>
<tr>
<td>Heart muscle</td>
<td>30 µg</td>
<td>8 µg</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>0 µg</td>
<td>0 µg</td>
</tr>
<tr>
<td>Brain</td>
<td>0 µg</td>
<td>0 µg</td>
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