The Effect of Thiosemicarbazide and other Urea Analogues on the Growth of *Tetrahymena geleii*

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**SUMMARY:** A number of urea analogues have been tested for inhibitory effects on the growth of *Tetrahymena geleii*. Semicarbazide and thiosemicarbazide appear to interfere with the formation of compounds containing carbonyl-groups. The inhibition can be partially overcome by increasing the concentration of pyridoxamine in the medium or completely overcome by adding keto-acids.

The thiosemicarbazones of β- and γ-pyridine carboxaldehyde and of p-acetaminobenzal are inhibitory but reversal of this inhibition was not possible with the compounds used.

Interest in thiosemicarbazide and the thiosemicarbazones for the treatment of tuberculosis was aroused by the report of Domagk, Behnisch, Mietzsch & Schmidt (1946). It was thought possible that the ciliate *Tetrahymena geleii* might serve as a test organism for further study of these compounds. This ciliate has the advantage of resembling the mammal in its metabolic requirements and therefore might be useful for the toxicity studies. On the other hand, its faster growth rate would be desirable as compared to the mycobacteria.

It was proposed therefore to determine the toxicity to *T. geleii* of thiosemicarbazide and a number of related substituted ureas, and if possible to obtain reversal of the toxic effects, in order to gain some insight into the metabolic system or systems involved.

**MATERIAL AND METHODS**

The organism used was *T. geleii* W. It was grown in medium A (Dewey, Parks & Kidder, 1950) or medium A in which Tween 80 (10 mg./ml.) was substituted for Tween 85. Appropriate omissions were made from these media. The experiments were carried out in such a way that the urea analogues were autoclaved separately from the remainder of the medium. The concentrations of these compounds were varied over a range so that half maximal inhibition of growth occurred at an approximately median concentration of the inhibitor (Kidder, Dewey & Parks, 1951). The growth responses were determined turbidimetrically, after 96 hr. incubation at 25° in tubes held in a slanted position, by use of a Lumetron photoelectric colorimeter with filter no. 650.

The compounds used, with the exception of hydroxylamine, are all related to urea (Fig. 1): ethyl carbamate, urea, thiourea, nitrourea, acetylene, acetylmethylurea, 3-thiosemicarbazide, semicarbazide, glucose thiosemicarbazone, pyruvic acid thiosemicarbazone, p-acetaminobenzal thiosemicarbazone, the β- and γ-pyridine carboxaldehyde thiosemicarbazones and pyridoxal thiosemicarbazone. The following compounds (Fig. 2), either because of their...
structural similarity or because of their known or possible metabolic functions, were tested for their ability to overcome inhibition: creatine, guanidoacetic acid, guanidine, aminoguanidine, pyruvic acid, α-ketoglutaric acid, γ-amino-butyric acid, pyridoxal, pyridoxal phosphate, pyridoxamine and nicotinamide.

\[
\begin{align*}
\text{NH}_2\text{OH} & \quad \text{Hydroxylamine} \\
\text{NH}_2\text{C}-\text{NH} & \quad \text{Urea} \\
\text{NH}_2\text{C}-\text{NH} & \quad \text{Nitrourea} \\
\text{NH}_2\text{C}-\text{O}-\text{CH}_3 & \quad \text{Ethyl carbamate} \\
\text{NH}_2\text{C}-\text{NH} & \quad \text{Thiourea} \\
\text{N} & \quad \text{Pyruvic semicarbazone} \\
\text{N} & \quad \text{Pyruvic thiosemicarbazone} \\
\end{align*}
\]

Fig. 1. Structures of thiosemicarbazide and related compounds tested for toxicity to \textit{T. gelii}.

RESULTS

Table 1 lists the compounds tested and gives the amount required for half-maximal inhibition in complete basal medium (which contains 0.1 μg. of pyridoxal HCl and of pyridoxamine HCl/ml.). It may be seen that the compounds most effective in the treatment of tuberculosis (Levaditi, Gerald,

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount required for ( \frac{1}{2} ) maximum inhibition (μg./ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>412</td>
</tr>
<tr>
<td>Nitrourea</td>
<td>123</td>
</tr>
<tr>
<td>Ethyl carbamate</td>
<td>5250</td>
</tr>
<tr>
<td>Thiourea</td>
<td>1120</td>
</tr>
<tr>
<td>Acetylurea</td>
<td>3100</td>
</tr>
<tr>
<td>Acetyl methylurea</td>
<td>2840</td>
</tr>
<tr>
<td>Hydroxylamine</td>
<td>12</td>
</tr>
<tr>
<td>Semicarbazide</td>
<td>126</td>
</tr>
<tr>
<td>Thiosemicarbazide</td>
<td>123</td>
</tr>
</tbody>
</table>

Table 1. \textit{Inhibition of T. geleii by urea and analogues}

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount required for ( \frac{1}{2} ) maximum inhibition (μg./ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvic semicarbazone</td>
<td>2000</td>
</tr>
<tr>
<td>Pyruvic thiosemicarbazone</td>
<td>600</td>
</tr>
<tr>
<td>Glucose thiosemicarbazone</td>
<td>1650</td>
</tr>
<tr>
<td>( \beta )-Pyridine carboxaldehyde thiosemi-</td>
<td>12</td>
</tr>
<tr>
<td>carbazone</td>
<td></td>
</tr>
<tr>
<td>( \gamma )-Pyridine carboxaldehyde thiosemi-</td>
<td>21</td>
</tr>
<tr>
<td>carbazone</td>
<td></td>
</tr>
<tr>
<td>( p )-Acetaminobenzal thiosemicarbazone</td>
<td>100</td>
</tr>
<tr>
<td>Pyridoxal thiosemicarbazone*</td>
<td>No inhibition</td>
</tr>
</tbody>
</table>

* Replaced vitamin B\(_6\) with 10–15 % activity.
Vaisman & Ray, 1950) were those which were the most inhibitory to *T. geleii*. No inhibition index can be given, of course, since the system in which these compounds are acting is unknown. The various members of the vitamin B₆ group (Table 2) were effective in reversing the inhibition caused by semicarbazide and thiosemicarbazide but not that of the other compounds tested. It

![Chemical Structures](image)

**Fig. 2.** Structures of various compounds tested for ability to overcome toxicity of thiosemicarbazide and related compounds for *T. gelii*.

should also be noted that thiosemicarbazide reversal was only partial. For example, after a certain concentration of pyridoxamine is reached, further increases in concentration give no further decrease of inhibition. Of the remainder of the compounds listed above as having been tested for their effect in reversing toxicity, only the keto-acids were active.

Hydroxylamine differed from the semicarbazides in that inhibition by it was overcome by the keto-acids but not by members of the vitamin B₆ group.
Table 2. Effects of $B_6$ vitamins on inhibition by various urea analogues

<table>
<thead>
<tr>
<th>B$_6$ vitamin additions (µg./ml.)</th>
<th>Urea analogue inhibitors</th>
<th>Amounts required for $\frac{1}{2}$ maximum inhibition (µg./ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nitro-</td>
<td>Ethyl Semi-</td>
</tr>
<tr>
<td></td>
<td>urea carbamate</td>
<td>carbamide</td>
</tr>
<tr>
<td>0.005</td>
<td>410</td>
<td>126</td>
</tr>
<tr>
<td>0.05</td>
<td>455</td>
<td>141</td>
</tr>
<tr>
<td>0.5</td>
<td>159</td>
<td>.</td>
</tr>
<tr>
<td>2.0</td>
<td>159</td>
<td>.</td>
</tr>
</tbody>
</table>

* = $\beta$-Pyridine carboxaldehyde thiosemicarbazone.
† = $\gamma$-Pyridine carboxaldehyde thiosemicarbazone.

It is also of interest that aminoguanidine, which has been suggested as being active in the metabolism of formaldehyde (Bernheim, 1950), was itself somewhat inhibitory and enhanced the inhibition produced by the semicarbazides. The inhibition due to the semicarbazones was not affected by increasing the concentration of $B_6$ compounds in the medium beyond the slight effect obtained by the increased growth in the presence of the larger amounts of the vitamin. The keto-acids were also ineffective in overcoming the inhibition caused by these compounds, as was nicotinamide, which may be considered to be a structural analogue of the $\beta$-pyridine carboxaldehyde thiosemicarbazone.

In the belief that the pyridine carboxaldehyde thiosemicarbazones might be acting as structural analogues of pyridoxal, the thiosemicarbazone of pyridoxal was prepared. This compound proved to be completely uninhibitory. When tested in the absence of vitamin $B_6$ it promoted growth. This was taken as evidence that the organism is capable of hydrolysing the thiosemicarbazone with consequent release of pyridoxal. The thiosemicarbazone of pyridoxal had 10-15% activity on a molar basis as compared to pyridoxal. The activity tended to decrease as the amount of thiosemicarbazone was increased. The thiosemicarbazone of $p$-acetaminobenzaldehyde was also tested for growth-inhibiting capacity, but because of its insolubility no precise figure was obtained.

**DISCUSSION**

Hydroxylamine was included in this series of compounds because, like the semicarbazides, it reacts with carbonyl groups. Whether the oximes formed are, like the semicarbazones, also inhibitory to growth was not determined. Hydroxylamine has, in addition, the ability to form hydroxamic acids with esters and anhydrides and, according to Borek, Grossowicz & Waelsch (1950),
Urea analogues and T. geleii

it may also produce inhibition by blocking transamidation reactions involving ammonia, asparagine and/or glutamine. The inhibition of bacterial growth due to interference with these reactions occurs at lower concentrations of the inhibitor than the interference with the metabolism of the carbonyl compounds. However, in the case of T. geleii, neither ammonia nor asparagine had any effect on the inhibition produced by hydroxylamine. Since the ciliate is said (Seaman, 1950) to produce no acetyl phosphate, the only known site of action for hydroxylamine is in carbonyl metabolism. In this regard it is of interest that acetaldehyde can function as the two-carbon fragment in the metabolism of T. geleii (Seaman, 1950) and that hydroxylamine inhibition is readily reversed by keto-acids. On the other hand, vitamin B₆ is without effect on the toxicity of hydroxylamine, in contrast to the case of the semicarbazides.

Pyridoxal phosphate is reported to participate in the decarboxylation of glutamic acid to γ-aminobutyric acid (Roberts & Frankel, 1951). Since the latter compound was ineffective in overcoming inhibition, it is apparent that thiosemicarbazide is not interfering with the activity of pyridoxal phosphate, per se, although it has not been demonstrated that γ-aminobutyric acid is a metabolic product of T. geleii. It is also true that creatine and its precursors have not yet been shown to be concerned in the metabolism of T. geleii and it is therefore unprofitable to speculate on their relationship to the semicarbazides. It is evident, however, that semicarbazide and related compounds are not acting as structural analogues of guanidine, guanidoacetic acid or creatine, since the latter substances were not active in overcoming inhibition.

It is apparent that the inhibitory effects of the semicarbazides in the growth of T. geleii are produced by the ability of these compounds to react with carbonyl groups, since pyruvic and α-ketoglutaric acids overcame the inhibition. Substances containing carbonyl groups appear to be produced during growth and to be necessary for growth. The addition of pyridoxamine in excess of the minimum amount required for growth stimulates the production of keto-acids by T. geleii (unpublished data). This effect, however, reaches a maximum, and further increases in pyridoxamine concentration do not influence keto-acid production. It may be assumed that the enzymes responsible for the production of such acids (e.g. deaminases) become saturated with the pyridoxamine coenzyme at this level. This would explain the failure of pyridoxamine to overcome completely the toxicity of the semicarbazides, while pyruvic and α-ketoglutaric acids are effective. This must be regarded as a non-specific effect due to the chemical interaction of these acids with the semicarbazides.

While these observations were responsible for the use of pyridoxamine in overcoming the toxicity of thiosemicarbazide in mice (Parks, Kidder & Dewey, 1951), the mechanism of this action in the latter case appears to be somewhat different, since the keto-acids are inactive. In view of this fact and of the fact that the toxicity of the semicarbazones is not overcome either by pyridoxamine or by keto-acids in T. geleii, it will be of interest to determine whether or not pyridoxamine will affect the toxicity of the thiosemicarbazones in mice.
We are indebted to Dr John Aeschlimann of the Hoffmann-LaRoche Co. for samples of p-acetaminobenzal thiosemicarbazone and of the two pyridine carboxaldehyde thiosemicarbazones. The pyridoxal phosphate was obtained through the courtesy of Dr Richard F. Phillips of Merck and Co.

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REFERENCES


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