The observation that salts of the alkaline earth metals reverse the inhibitory effects of atabrine in the growth of Escherichia coli (1) suggested, as one possibility, that the inhibitor acts by forming complexes with metal-catalyzed enzyme systems. The effect of atabrine on yeast carboxylase was therefore investigated, but the results show no evidence for complex formation involving atabrine and this metallo-enzyme. Instead, the investigation has disclosed that atabrine competitively inhibits the dephosphorylation of cocarboxylase by a yeast phosphatase and, further, that the drug inhibits the synthesis of thiamine by yeast cultures.

Methods

A batch of brewers’ yeast which had been washed extensively in water, then dried and stored at room temperature, was used as the source of carboxylase. Atiozymase was prepared daily from this lot of yeast by rapidly washing 1 gm. with six successive 45 ml. portions of 0.1 M K₂HPO₄. A final washing was carried out with 25 ml. of 0.067 M KH₂PO₄. The temperature of the wash solutions was maintained at 10°.

Cocarboxylase concentrations were determined manometrically by the customary Warburg procedures. The components of the assay system and the order of the additions to the vessels were as follows: (1) Mn⁺⁺, \(4.4 \times 10^{-4} \text{ M}\); (2) standard cocarboxylase solution or sample; (3) thiamine, \(10^{-4} \text{ M}\) or atabrine, \(10^{-3} \text{ M}\) (see the text); (4) phosphate buffer, pH 6.1, \(2.7 \times 10^{-2} \text{ M}\); (5) atiozymase, 50 mg. in solution (4); (6) sodium pyruvate, \(2.7 \times 10^{-3} \text{ M}\); total volume 2.5 ml.; temperature 30°. The pyruvate was added after temperature equilibrium was attained and the CO₂ evolved was measured after 15 minutes.

Thiamine concentrations were determined by a thiochrome procedure of Papageorge and Lamar (2). Thiamine was extracted from cell suspensions by boiling for 1 minute in 0.05 N HCl.

Torula utilis, ATCC 9255, was grown in the mineral salts-glucose medium of Gray and Tatum (3). The inoculum employed was the growth from 10 ml. of 1 per cent Bacto-peptone incubated at 30° for 24 hours.
The cells were washed twice by centrifugation with saline and then added to 500 ml. of the mineral salts medium.

**Results**

The effect of the addition of atabrine to the carboxylase system was first investigated. Examination of Fig. 1 shows the dependence of the test system on the presence of cocarboxylase and manganese. In the absence of either of the two factors carboxylase activity was negligible. Under the conditions indicated about $0.8 \times 10^{-8}$ mole of cocarboxylase and $1.1 \times 10^{-8}$ mole of Mn$^{++}$ were required to saturate the system.

![Image](http://www.jbc.org/)

**Fig. 1. Atabrine and carboxylase activity.** Components of reaction mixture: 100 mg. of alkaline-washed yeast; phosphate $2.7 \times 10^{-3}$ M, pH 6.1; cocarboxylase $8 \times 10^{-7}$ M; Mn$^{++}$ $4.4 \times 10^{-4}$ M; sodium pyruvate $2.7 \times 10^{-4}$ M; volume 2.5 ml.; temperature 30°. Curve A, system lacks Mn$^{++}$; Curve B, system lacks cocarboxylase; Curve C, complete reaction mixture; Curve D, complete reaction mixture + $8 \times 10^{-4}$ M atabrine.

These points are brought out to indicate that almost complete dissociation of the enzyme had occurred during the washing procedure and thus the drug would be given a favorable opportunity to interfere with the reconstitution of the carboxylase system. However, with the addition of atabrine ($8 \times 10^{-4}$ M) the rate increased more than 2-fold (Fig. 1).

This stimulation of carboxylase activity by atabrine was now investigated. In the absence of added cocarboxylase, atabrine was without effect and it became apparent that the action of atabrine was similar to that of thiamine first reported by Ochoa (4). Westenbrink *et al.* (5) showed that the stimulatory effects of thiamine were due to the inhibition of a yeast phosphatase which dephosphorylates cocarboxylase.
The atiozymase preparation employed was a potent source of phosphatase. This is indicated in Tables I and II, which illustrate the rate of disappearance of cocarboxylase and the rate of formation of free thiamine from cocarboxylase by this atiozymase preparation. The initial concentration of cocarboxylase was reduced by about 50 per cent in 5 minutes.

The effect of atabrine on the destruction of cocarboxylase by the washed yeast preparation is indicated in Table III. With atabrine at a concentration of $10^{-4} \text{ M}$, the inhibition of phosphatase activity is almost complete. A slight effect is evident at $10^{-6} \text{ M}$ under the test conditions employed.
A comparison of the effects of thiamine and atabrine on the activity of the carboxylase preparation is shown in Table IV. With the concentration of cocarboxylase employed, $4 \times 10^{-7} \, \text{M}$, maximum activity was obtained with thiamine at about $10^{-4} \, \text{M}$; with atabrine, at about $10^{-3} \, \text{M}$.

**Table IV**

*Comparison of Thiamine and Atabrine Effects on Carboxylase Activity*

<table>
<thead>
<tr>
<th>Concentration</th>
<th>0 M</th>
<th>$10^{-7}$ M</th>
<th>$10^{-6}$ M</th>
<th>$10^{-5}$ M</th>
<th>$10^{-4}$ M</th>
<th>$10^{-3}$ M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamine</td>
<td>105</td>
<td>139</td>
<td>166</td>
<td>231</td>
<td>243</td>
<td>245</td>
</tr>
<tr>
<td>Atabrine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values in c.mm. of CO$_2$ per 15 minutes. Alkaline-washed yeast 50 mg.; phosphate $2.7 \times 10^{-2} \, \text{M}$, pH 6.1; Mn$^{+2}$ $4.4 \times 10^{-4} \, \text{M}$; Na pyruvate $2.7 \times 10^{-2} \, \text{M}$; cocarboxylase $4 \times 10^{-7} \, \text{M}$; volume 2.5 ml.; temperature, 30$^\circ$.

![Fig. 2. Velocity of yeast phosphatase action in presence of several concentrations of cocarboxylase and atabrine. Curves A, B, and C represent mixtures containing 0, $10^{-4}$, and $5 \times 10^{-4}$ M atabrine. S represents molar cocarboxylase $\times 10^{-4}$; V represents moles $\times 10^{-8}$ cocarboxylase inactivated in 10 minutes.](image)

Thus thiamine seems to be about 10 times more effective than atabrine as an inhibitor of the phosphatase activity. The nature of the atabrine inhibition of phosphatase action appears to be that of competition. This is indicated by the data plotted in Fig. 2. The reciprocal of the reaction velocity is plotted against substrate concent-
tration in the presence and absence of atabrine. The progressive increase in slope with increase in concentration of atabrine, together with the apparent common intercept, indicates that the inhibition is competitive in nature (6).

The antimalarial drug, chloroquine, is more potent than atabrine as an inhibitor of the yeast phosphatase which splits cocarboxylase (Table V). Its effect was evident at a concentration of $10^{-6}$ M. The basic drugs, quinine, paludrine, and stilbamidine, had little or no effect. In a study of cholinesterase activity, Wright and Sabine (7) obtained somewhat similar results. They report that atabrine and chloroquine are far more effective than quinine or paludrine as inhibitors of cholinesterase. In examining Table V, it must be borne in mind that an inhibition of the cocarboxylase-cleaving mechanism is indicated by increased carboxylase activity.

**Table V**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Atabrine</th>
<th>Quinine</th>
<th>Paludrine</th>
<th>Chloroquine</th>
<th>Stilbamidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>52</td>
<td>52</td>
<td>52</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>$10^{-6}$</td>
<td>62</td>
<td>52</td>
<td>54</td>
<td>93</td>
<td>57</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>84</td>
<td>50</td>
<td>51</td>
<td>157</td>
<td>57</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>178</td>
<td>55</td>
<td>45</td>
<td>198</td>
<td>45</td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>237</td>
<td>69</td>
<td>50</td>
<td>237</td>
<td>62</td>
</tr>
</tbody>
</table>

Values in c.mm. of CO₂ per 15 minutes. The test conditions are as in Table IV.

Massart et al. (8) have shown that cations can prevent the combination of basic dyes with yeast cell enzymes. However, Ca⁺⁺, which completely negates the action of atabrine in the growth of *Escherichia coli* (9), was without effect in reducing the toxicity of the drug for the yeast phosphatase. These negative results were obtained with Ca⁺⁺ at a level of $6 \times 10^{-3}$ M and atabrine at concentrations of $10^{-3}$ and $8 \times 10^{-5}$ M.

Yeast carboxylase itself is relatively insensitive to the action of the antimalarial. A 20 per cent reduction of the maximum activity was observed with atabrine at a concentration of $1.2 \times 10^{-2}$ M.

The effect of atabrine on yeast growth activity with respect to crop yield and thiamine and cocarboxylase cell content was next examined. The yeast *Torula utilis* was employed in these studies. This organism grows well in a mineral salts-glucose medium and its growth is markedly stimulated by the addition of thiamine. An examination of freshly harvested cells (18 hours old) grown without added thiamine showed that they contained less than 0.2 γ of free thiamine per gm. (dry basis). Thus
the cellular thiamine is almost completely in the form of cocarboxylase (Table VI).

The effects of atabrine are shown in Table VI. In the absence of added thiamine, increasing concentrations of atabrine progressively inhibited the cell yield and, associated with this, a progressive decrease in cocarboxylase content occurred. In the presence of added thiamine, the crop yield was somewhat more than doubled and the cocarboxylase content increased 8- to 10-fold. Again, progressive decreases in cell yield were observed with increasing atabrine concentrations. However, no de-

**Table VI**

<table>
<thead>
<tr>
<th>Atabrine, $M$</th>
<th>Yield, $ml$</th>
<th>Cocarboxylase, $\gamma$ per gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>No thiamine</td>
<td>0</td>
<td>35.1</td>
</tr>
<tr>
<td>$5 \times 10^{-6}$</td>
<td>0.19</td>
<td>45.0</td>
</tr>
<tr>
<td>Atabrine, $M$</td>
<td>0</td>
<td>$5 \times 10^{-6}$</td>
</tr>
<tr>
<td>Yield, $ml$</td>
<td>0.20</td>
<td>42.5</td>
</tr>
<tr>
<td>Cocarboxylase, $\gamma$ per gm</td>
<td>45.0</td>
<td>$3 \times 10^{-6}$</td>
</tr>
<tr>
<td>Atabrine, $M$</td>
<td>0</td>
<td>0.36</td>
</tr>
<tr>
<td>Yield, $ml$</td>
<td>0.16</td>
<td>0.42</td>
</tr>
<tr>
<td>Cocarboxylase, $\gamma$ per gm</td>
<td>37</td>
<td>296</td>
</tr>
</tbody>
</table>

The yields are expressed in terms of packed cell paste recovered on centrifugation from 500 ml. of mineral salts medium after 18 hours incubation at 30°. Cocarboxylase concentrations are expressed in micrograms per gm. of dry cell weight.

creases in cocarboxylase content occurred. Apparently atabrine interference with thiamine metabolism in the growth of Torula utilis involves a step in the synthesis of thiamine and not the conversion of the latter to cocarboxylase.

**DISCUSSION**

Because (a) atabrine bears some structural relationship to riboflavin and (b) the drug can interfere with reactions involving flavin nucleotides, some support has been given to the concept that a flavin inhibitor antagonism exists which is specific for atabrine (10-13). However, there
are objections to accepting the flavin-atabrine antagonism as specific in nature. Hellerman et al. (14) have already pointed out some inconsistencies. The data reported now dealing with atabrine and thiamine relationships indicate that the growth-inhibitory action of atabrine is not limited to interference of systems involving flavin derivatives. In this connection it may be noted that riboflavin was no more effective than thiamine or several other B vitamins in reversing the bacteriostatic action of atabrine in the growth of *Escherichia coli* (15).

It seems unlikely that the inhibition of cocarboxylase dephosphorylation observed in dried yeast preparations plays a rôle in the inhibition of cell growth. One would expect that, if the inhibition of cocarboxylase cleavage were associated with inhibition of yeast growth, increases in cellular cocarboxylase would occur in cells whose growth was limited by the presence of atabrine. However, cells grown in the presence of atabrine have cocarboxylase concentrations below or equal to that of normal cells.

Hegsted et al. (16) have reported that atabrine exerts a thiamine-sparing action in the growth of rats fed a thiamine-deficient diet. A somewhat similar picture is presented by the action of atabrine as it affects carboxylase activity in dried yeast. In this instance, however, atabrine exerts a "cocarboxylase sparing" action by inhibiting inactivation of the coenzyme. It may very well be that the sparing action of atabrine in the rat results from its interference with cellular mechanisms which normally convert the enzymatically active form of thiamine, diphosphothiamine, to inactive forms.

**SUMMARY**

1. Atabrine competitively inhibits the dephosphorylation of cocarboxylase by a dried yeast preparation. Chloraquine is a more effective inhibitor than atabrine, whereas quinine, paludrine, and stilbamidine are without effect.

2. Atabrine inhibits the synthesis of thiamine by growing cells of *Torula utilis*. At concentrations causing growth inhibition the drug is without effect on the conversion of thiamine to diphosphothiamine.

3. Thiamine in freshly harvested cells of *Torula utilis* is almost completely in the form of cocarboxylase.

4. It is concluded that the atabrine inhibition of cocarboxylase dephosphorylation observed in dried yeast preparations does not have a rôle in accounting for the growth-inhibitory effects of the drug.

**BIBLIOGRAPHY**

INTERACTIONS OF ATABRINE, THIAMINE, AND COCARBOXYLASE
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