THE BIOCHEMISTRY OF THE MALARIA PARASITE*

III. THE EFFECTS OF QUININE AND ATABRINE ON GLYCOLYSIS

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In Paper II (1) it was shown that the formation of lactic acid from glucose by the malaria parasite is a process similar to the phosphorylating glycolysis of yeast and vertebrate muscle. Quinine and atabrine have been found to inhibit the glycolysis (2) as well as the oxygen uptake (2-4) of the parasite. Since glycolysis is a preliminary stage in the oxidation of glucose under aerobic conditions and is the only path for the utilization of glucose anaerobically, it was believed that an investigation of the effects of antimalarial drugs on this process might aid in understanding the mode of action of these drugs.

The present paper describes the action of quinine and atabrine on the phosphorylation of glucose and the dehydrogenation of 3-phosphoglyceraldehyde and lactic acid by preparations of the avian parasite Plasmodium gallinaceum. Comparable experiments with glycolytic enzymes from yeast and mammalian muscle are also reported.

Methods and Materials

Inorganic phosphate was determined by the method of Gomori (5) on trichloroacetic acid filtrates. Quinine interferes with the estimation of phosphate by the molybdenum blue method by forming a precipitate with phosphomolybdic acid, and atabrine interferes because of its deep yellow color. Both difficulties can be avoided by extracting the free bases from alkaline solution with an immiscible solvent. The solution to be analyzed for phosphate is made alkaline to phenolphthalein, extracted twice with an equal volume of carbon tetrachloride, and clarified by centrifugation.

Solutions of quinine and atabrine for use in the enzyme experiments were prepared by neutralizing solutions of the dihydrochlorides with 1.5 equivalents of sodium bicarbonate. The final concentration of the neutralized solutions was not made greater than 0.01 M; otherwise the bases did not remain in solution long enough to permit their being pipetted into the reaction mixtures.

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Adenosine triphosphate, fructose-1, 6-diphosphate, and diphosphopyridine nucleotide were prepared as described in Paper II (1). The glycogen and adenylic acid were commercial preparations. Potassium glucose-1-phosphate was obtained essentially by the method of Hanes (6). In our experience, after removal of barium with sulfuric acid and neutralization with potassium hydroxide, the potassium glucose-1-phosphate did not precipitate on addition of an equal volume of alcohol. It could be obtained in crystalline form by adding more alcohol to the supernatant and allowing it to stand in the cold. Phosphoglyceric acid was prepared according to Neuberg and Kobel (7). Phosphopyruvic acid was synthesized by a modification of the method of Kiessling (8) suggested by Dr. Gerhardt Schmidt. Adenosine diphosphate was prepared from adenosine triphosphate by the use of yeast hexokinase (9).

The preparation of enzyme extracts from malaria parasites (Plasmodium gallinaceum) is described in Paper II (1). The enzymes from yeast and muscle are described in connection with the particular reaction for which they were used.

Results

Effects of Quinine and Atabrine on Glycolysis in Preparations from Malaria Parasites

Phosphorylation of Glucose—The effect of quinine and atabrine on the phosphorylation of glucose by hemolysates of normal and parasitized chicken red blood cells was studied with the manometric system described in Paper II (1). The results of several experiments are summarized in Table I. Quinine in concentrations from 0.0001 to 0.002 M inhibited the phosphorylation of glucose in hemolysates of normal chicken red cells but caused only a slight inhibition in hemolysates of parasitized red cells. The inhibition in the latter case was not greater than would be expected from inhibition of that fraction of the total hexokinase activity (about one-fourth) derived from the enzymes of the red cells themselves. However, atabrine in the same concentrations as quinine caused an equal inhibition of the phosphorylation of glucose, in hemolysates of both normal and parasitized erythrocytes.

3-Phosphoglyceraldehyde Dehydrogenase—The effects of quinine and atabrine on the enzyme 3-phosphoglyceraldehyde dehydrogenase were studied in a system in which the over-all process was that described by Equation 1. Three enzymatic reactions are involved in this process:

\[ \text{Fructose-1, 6-diphosphate} + \text{pyruvate} \rightarrow \]
\[ \text{3-phosphoglycerate} + \text{H}^+ + \text{lactate} + \text{dihydroxyacetone phosphate} \]  

(1) the splitting of fructose-1, 6-diphosphate to 3-phosphoglyceraldehyde

1 Schmidt, G., personal communication.
and dihydroxyacetone phosphate (catalyzed by the enzyme aldolase); (2) the oxidation of 3-phosphoglyceraldehyde by diphosphopyridine nucleotide in the presence of arsenate to form 3-phosphoglyceric acid and reduced diphosphopyridine nucleotide (catalyzed by the enzyme 3-phosphoglyceraldehyde dehydrogenase); and (3) the reoxidation of the reduced diphosphopyridine nucleotide by pyruvate to form diphosphopyridine nucleotide and lactate (catalyzed by the enzyme lactic dehydrogenase) (see the discussion of the oxidation-reduction reactions in Paper II (1)). An excess of purified preparations of the enzymes aldolase and lactic dehydrogenase was added; therefore the rate of the over-all process was determined by the activity of the 3-phosphoglyceraldehyde dehydrogenase.

**Table I**

**Effect of Quinine and Atabrine on Hexokinase Activity of Red Blood Cell Hemolysates**

The sample contained 0.028 M NaHCO₃, 0.004 M MgSO₄, 0.028 M KF, 0.012 M glucose, 0.0036 M adenosine triphosphate (added from the side arm after equilibration), 1.4 cc. of hemolysate of normal chicken red cells or 0.7 cc. of hemolysate of parasitized red cells, and the concentrations of quinine and atabrine indicated below, in a total volume of 2.5 cc. Warburg manometers; gas phase, 5 per cent CO₂-95 per cent N₂; temperature 39°.

<table>
<thead>
<tr>
<th>Concentration of inhibitor</th>
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<tbody>
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<tr>
<td>M</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0.0001</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0.0005</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.002</td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Per cent inhibition by Quinine</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Parasitized</td>
<td>Normal</td>
<td>Parasitized</td>
</tr>
<tr>
<td>0.0001</td>
<td>6</td>
<td>4</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>0.0005</td>
<td>18</td>
<td>4</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>0.001</td>
<td>23</td>
<td>8</td>
<td>31</td>
<td>26</td>
</tr>
<tr>
<td>0.002</td>
<td>43</td>
<td>10</td>
<td>40</td>
<td>39</td>
</tr>
</tbody>
</table>

Aldolase was purified according to Herbert *et al.* (10) through the stage of the first fractionation with ammoniacal ammonium sulfate. Lactic dehydrogenase was prepared from beef heart by following the method of Straub (11) through the second ammonium sulfate precipitation. Since acid is formed, the reaction was followed by measuring the evolution of carbon dioxide from a bicarbonate buffer containing the test system. The complete test system contained bicarbonate, arsenate, fluoride, fructose-1,6-diphosphate, pyruvate, and the three enzymes aldolase, 3-phosphoglyceraldehyde dehydrogenase, and lactic dehydrogenase. If only aldolase and lactic dehydrogenase were added, no acid was formed. Therefore these two preparations were free from 3-phosphoglyceraldehyde dehydrogenase activity. When a preparation containing 3-phosphoglyceraldehyde dehydrogenase was added, in addition to the other two enzymes, acid was formed at a rate which was proportional to the amount of dehydrogenase.
added and was constant until at least one-third of the fructose-1,6-diphosphate had been used. Extracts of parasite material (1) as well as preparations made from rabbit skeletal muscle were effective as sources of 3-phosphoglyceraldehyde dehydrogenase.

The effect of quinine and atabrine on the enzyme 3-phosphoglyceraldehyde dehydrogenase extracted from parasite material was investigated by the use of the system just described. In concentrations up to 0.002 M neither drug inhibited this enzyme significantly. These results are shown in Table II.

**Lactic Dehydrogenase**—The effect of quinine and atabrine on the enzyme lactic dehydrogenase in extracts of parasite material was studied by means of the colorimetric technique of Haas (12), as described in Paper II (1). The results of two experiments are shown in Table III. Both drugs inhibited the enzyme, but atabrine was considerably more effective than quinine.

**Effect of Quinine and Atabrine on Yeast Hexokinase**

The enzyme hexokinase in yeast catalyzes the transfer of one phosphate group from adenosine triphosphate to glucose, to form glucose-6-phosphate and adenosine diphosphate (9). This same reaction is apparently the first step in the phosphorylation of glucose by the malaria parasite also (1). Hexokinase was prepared from bakers' yeast according to Colowick and Kalckar (9), and the reaction was studied manometrically in a test system
similar to theirs. Determination of the labile pyrophosphate groups of adenosine triphosphate (phosphate hydrolyzed by 1 M H₂SO₄ in 10 minutes at 100° (13)) showed that the formation of acid was accompanied by a decrease in pyrophosphate groups and the formation of a more stable phosphate compound.

**Table III**

*Effect of Quinine and Atabrine on Lactic Dehydrogenase in Parasite Extracts*

The samples contained 0.02 M phosphate at pH 7.4, 54 g of sodium dichlorophenol indophenol, 0.1 M lithium dl-lactate, 0.00003 M diphosphopyridine nucleotide, 0.4 cc. of an extract of parasite material, and the concentrations of quinine and atabrine indicated below, in a total volume of 5.0 cc.; temperature 20°. The rate of reaction was measured as the increase in percentage transmission per unit time, read in an Evelyn photoelectric colorimeter with Filter 620.

<table>
<thead>
<tr>
<th>Concentration of inhibitor (M)</th>
<th>Per cent inhibition by</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Quinine</td>
</tr>
<tr>
<td>0.0001</td>
<td>5</td>
</tr>
<tr>
<td>0.0003</td>
<td>7</td>
</tr>
<tr>
<td>0.001</td>
<td>16</td>
</tr>
</tbody>
</table>

**Table IV**

*Effect of Quinine and Atabrine on Yeast Hexokinase*

The samples contained 0.02 M NaHCO₃, 0.01 M MgSO₄, 0.02 M glucose, 0.005 M adenosine triphosphate (tipped in from the side arm after equilibration), hexokinase preparation containing 1.0 mg. of protein, and the concentrations of quinine and atabrine indicated below, in a total volume of 2.0 cc. Warburg manometers; gas phase, 5 per cent CO₂-95 per cent N₂; temperature 30°. The rate of reaction was about 140 microliters of CO₂ per hour.

<table>
<thead>
<tr>
<th>Concentration of inhibitor (M)</th>
<th>Per cent inhibition by</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Quinine</td>
</tr>
<tr>
<td>0.0005</td>
<td>6</td>
</tr>
<tr>
<td>0.001</td>
<td>16</td>
</tr>
<tr>
<td>0.002</td>
<td>23</td>
</tr>
</tbody>
</table>

The average results of seven experiments with quinine and the results of a single experiment with atabrine are given in Table IV. Both quinine and atabrine are seen to inhibit the action of yeast hexokinase.

**Effect of Quinine on Glycolysis by Muscle Enzymes**

*Acid Formation from Glycogen*—The over-all reaction of glycolysis in rabbit muscle extracts is the formation of lactic acid from glycogen. Since
acid is produced, the course of the reaction can be followed by measuring
the evolution of carbon dioxide from a bicarbonate buffer containing the
test system (14). The system used contained 0.028 M NaHCO₃, 0.007 M
phosphate at pH 7.4, 9 mg. of glycogen, and 50 mg. of powder obtained by
lyophilizing an aqueous extract of rabbit skeletal muscle (14), in a volume
of 3.0 cc. The samples were placed in Warburg manometers under an
atmosphere of 5 per cent CO₂-95 per cent N₂, at a temperature of 35°,
and the glycogen was tipped in from the side arm after equilibration.
About 300 microliters of CO₂ per hour were evolved.

Quinine consistently inhibited the formation of acid in this system. The
average results from several experiments indicated an inhibition of 8 per
cent at a quinine concentration of 0.0005 M, of 14 per cent at 0.001 M,
and of 31 per cent at 0.002 M. The inhibition was greatest when the enzyme
was incubated with quinine before the reaction was started. When the
quinine was tipped in at the same time as the glycogen, smaller inhibitions
were observed.

**Phosphorylase**—The phosphorylation of glycogen in muscle extracts
occurs in the manner indicated in Equation 2 (15). Phosphorylase, the

\[
(C_{6}H_{10}O_{5})_{n} + nHPO_{4}^{-} \rightarrow nC_{6}H_{12}O_{10}P_{0}^{-}
\]

enzyme catalyzing this reaction, was prepared from rabbit muscle extract
according to Green, Cori, and Cori (16). It was not crystalline but was
active and free from phosphoglucomutase activity (conversion of glucose-
1-phosphate to glucose-6-phosphate).

The forward reaction, formation of glucose-1-phosphate, was studied
in a system containing the enzyme phosphorylase, inorganic phosphate,
glycogen, adenylic acid (as coenzyme), and reduced glutathione (to insure
maximum activity of the enzyme). The reaction was followed by deter-
mining the decrease in inorganic phosphate. The results of experiments
on the effect of quinine on the phosphorylation of glycogen are given in
Table V. Quinine markedly inhibited this reaction. The inhibition was
proportional to the concentration of quinine and decreased with time.

The reverse reaction, formation of polysaccharide from glucose-1-
phosphate, was studied in a system like that of Cori and Cori (17). The
process was followed by measuring the increase in inorganic phosphate.
A marked inhibition by quinine was observed, just as with the forward
reaction. The degree of inhibition was proportional to the concentration
of quinine and decreased with time. These results are illustrated in
Table VI.

Since these experiments measured the rate at which the reactions
approached an equilibrium, a decrease in inhibition with longer periods of
time was to be expected.
**Phosphoglucomutase**—The enzyme phosphoglucomutase catalyzes the attainment of an equilibrium between glucose-1-phosphate and glucose-6-phosphate (18). The enzyme used in these experiments was prepared according to Colowick and Sutherland (19), and the test system was similar to theirs.

The results of a typical experiment on the effect of quinine on phosphoglucomutase are shown in Table VII. Quinine inhibited the conversion

<table>
<thead>
<tr>
<th>Time</th>
<th>Decrease in inorganic P</th>
<th>Per cent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No quinine</td>
<td>0.001 M quinine</td>
</tr>
<tr>
<td></td>
<td>γ per cc.</td>
<td>γ per cc.</td>
</tr>
<tr>
<td>20 min.</td>
<td>180</td>
<td>90</td>
</tr>
<tr>
<td>40 min.</td>
<td>250</td>
<td>190</td>
</tr>
</tbody>
</table>

**TableVI**

**Effect of Quinine on Polysaccharide Formation from Glucose-1-Phosphate**

The samples contained 0.05 M glycero- and phosphate at pH 7.2, 1 per cent glycogen, phosphorylase preparation containing 6 mg. of protein, and the concentrations of quinine indicated below, in a total volume of 4 cc.; temperature 25°.

<table>
<thead>
<tr>
<th>Time</th>
<th>Increase in inorganic P</th>
<th>Per cent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No quinine</td>
<td>0.001 M quinine</td>
</tr>
<tr>
<td></td>
<td>γ per cc.</td>
<td>γ per cc.</td>
</tr>
<tr>
<td>5 min.</td>
<td>132</td>
<td>76</td>
</tr>
<tr>
<td>10 min.</td>
<td>252</td>
<td>161</td>
</tr>
</tbody>
</table>

of glucose-1-phosphate to glucose-6-phosphate, but to a variable extent.

**Phosphorylation of Fructose-6-phosphate**—Fructose-6-phosphate is converted to fructose-1,6-diphosphate by a transfer of phosphate from adenosine triphosphate, catalyzed by the enzyme phosphohexokinase (20). Since this transfer results in the liberation of acid, rate studies can conveniently be made by measuring the evolution of carbon dioxide when the reaction takes place in a bicarbonate buffer. The reaction mixture used
in these experiments contained 0.02 M NaHCO₃, 0.006 M MgSO₄, 0.0012 M sodium iodoacetate, 0.012 M potassium glucose-1-phosphate, 0.004 M adenosine triphosphate (tipped in from the side arm after equilibration), and a dialyzed extract equivalent to 80 mg. of an acetone powder of rabbit muscle (21), in a total volume of 2.5 cc. The gas phase in the Warburg manometers was 5 per cent CO₂-95 per cent N₂, and the temperature was 37°. The iodoacetate prevented liberation of acid by further reaction of the fructose-1,6-diphosphate formed. Glucose-1-phosphate was used as the substrate instead of fructose-6-phosphate because of its availability. During the preliminary incubation before the adenosine triphosphate was tipped in and during the subsequent experimental period, the glucose-1-phosphate was converted to glucose-6-phosphate and the latter substance rearranged to fructose-6-phosphate by the enzymes phosphoglucomutase and phosphohexoisomerase, which were present in the acetone powder extract. Since the phosphate groups of fructose-6-phosphate and fructose-1,6-diphosphate are less easily hydrolyzable than the pyrophosphate groups of adenosine diphosphate and triphosphate, the reaction can be followed approximately by measuring the decrease in pyrophosphate (phosphate liberated by hydrolysis with 1 N H₂SO₄ for 10 minutes at 100° (13)). Experiments in which organic phosphate fractions were followed showed that acid production was accompanied by a decrease in pyrophosphate groups. Control manometric experiments demonstrated that no acid was formed from adenosine triphosphate in the absence of glucose-1-phosphate, from glucose-1-phosphate in the absence of adenosine triphosphate, or from fructose-1,6-diphosphate. The rate of reaction in the complete system was about 160 microliters of CO₂ in 30 minutes.

Quinina in concentrations from 0.0005 to 0.002 M caused no inhibition of the phosphorylation of fructose-6-phosphate.

**Aldolase**—The enzyme aldolase catalyzes the splitting of fructose-1,6-
diphosphate to 3-phosphoglyceraldehyde and dihydroxyacetone phosphate. This enzyme was purified according to Herbert et al. (10) through the stage of heat coagulation, and the reaction was studied in a test system similar to the one described by these authors. Since the reaction reaches an equilibrium unless the products are removed, cyanide was added to bind the triose phosphate. Samples contained 0.05 M borate buffer at pH 7.3, 0.1 M HCN at pH 7.3, 0.012 M fructose-1,6-diphosphate, and 0.3 cc. of enzyme solution, in a total volume of 4.0 cc. These samples were incubated at 37°. Since the phosphate groups of both molecules of triose phosphate are hydrolyzed by treatment with 1 N NaOH for 20 minutes at room temperature while those of fructose-1,6-diphosphate are not

Table VIII

Effect of Quinine and Atabrine on 3-Phosphoglyceraldehyde Dehydrogenase from Rabbit Muscle

The samples contained 0.03 M NaHCO₃, 0.005 M Na₂HAsO₄, 0.03 M NaF, 0.02 M sodium pyruvate, 0.00037 M diprophosphorylde nucleotide, 0.005 M fructose-1,6-diphosphate (tipped in from the side arm after equilibration), aldolase preparation containing 1 mg. of protein, lactic dehydrogenase preparation containing 4 mg. of protein, dialyzed extract equivalent to 5 mg. of acetone powder of rabbit muscle extract (21), and the concentrations of quinine and atabrine indicated below, in a total volume of 2.5 cc. Warburg manometers; gas phase, 5 per cent CO₂-95 per cent N₂; temperature 30°.

<table>
<thead>
<tr>
<th>Concentration of inhibitor</th>
<th>Activity of dehydrogenase</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Quinine</td>
</tr>
<tr>
<td></td>
<td>microliters CO₂ per 5 min.</td>
</tr>
<tr>
<td>0</td>
<td>10.9</td>
</tr>
<tr>
<td>0.0001</td>
<td>10.7</td>
</tr>
<tr>
<td>0.0003</td>
<td>9.9</td>
</tr>
<tr>
<td>0.001</td>
<td>10.0</td>
</tr>
<tr>
<td>0.002</td>
<td>10.4</td>
</tr>
</tbody>
</table>

affected, the reaction was followed by determining the increase in alkali-labile phosphate (22). In 5 minutes about 220 γ of alkali-labile P per cc. were formed.

Quinine in concentrations from 0.0005 to 0.002 M caused no significant inhibition of the enzyme aldolase.

3-Phosphoglyceraldehyde Dehydrogenase—The effect of quinine and atabrine on the enzyme 3 phosphoglyceraldehyde dehydrogenase from rabbit muscle was investigated by means of the test system involving Reaction 1, which was used to study the activity of the same enzyme in parasite extracts. As in the latter case neither quinine nor atabrine caused a significant inhibition of 3-phosphoglyceraldehyde dehydrogenase. The results of two experiments are given in Table VIII.
Lactic Dehydrogenase—The action of quinine and atabrine on the enzyme lactic dehydrogenase obtained from beef heart was studied with the aid of the colorimetric technique of Haas (12), as described in Paper II (1). The enzyme was purified by the method of Straub (11) through the stage of the second ammonium sulfate precipitation. In the test system the enzyme showed no activity in the absence of lactate or diphosphopyridine nucleotide.

Lactic dehydrogenase from beef heart was strongly inhibited by atabrine but was not significantly affected by quinine. The experimental data are given in Table IX. The results are similar to those obtained with lactic dehydrogenase from parasite extracts.

### Table IX

**Effect of Quinine and Atabrine on Lactic Dehydrogenase from Beef Heart**

The samples contained 0.03 M phosphate buffer at pH 7.2, 54 γ of sodium dichlorophenol indophenol, 0.1 M lithium d-lactate, 0.00003 M diphosphopyridine nucleotide, lactic dehydrogenase preparation containing 1.1 mg. of protein, and the concentrations of quinine and atabrine indicated below, in a total volume of 5.0 cc.; temperature 25°. The rate of reaction was measured as the increase in percentage transmission (ΔG) per unit time, read in an Evelyn photoelectric colorimeter with Filter 620. Control samples showed a value for ΔG of 16.0 in 4 minutes.

<table>
<thead>
<tr>
<th>Concentration of inhibitor</th>
<th>Per cent inhibition by</th>
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<td></td>
<td>Quinine</td>
</tr>
<tr>
<td>0.0001</td>
<td>2</td>
</tr>
<tr>
<td>0.0003</td>
<td>0</td>
</tr>
<tr>
<td>0.001</td>
<td>6</td>
</tr>
</tbody>
</table>

Rearrangement of 3 Phosphoglycerate to Phosphopyruvate—The first steps in the breakdown of 3-phosphoglycerate in muscle extracts are rearrangement to 2-phosphoglycerate and dehydration of the latter substance to phosphopyruvate (23, 24) (Equation 3). The conversion of 3-phosphoglycerate to phosphopyruvate can be followed by determining the increase in phosphopyruvate phosphate, which is released on oxidation of the phosphopyruvate by hypoiodite (23). The reaction mixture used in studying this process contained 0.05 M veronal buffer at pH 8.2, 0.005 M MgSO₄, 0.016 M 3-phosphoglycerate, and dialyzed extract equivalent to 4 or 5 mg. of
acetone powder of rabbit muscle (21), in a total volume of 1.0 cc. The samples were incubated for 10 minutes at 37°. Control samples formed about 125 γ of phosphopyruvate P per cc.

Quinine in concentrations from 0.0002 to 0.002 M did not inhibit the conversion of 3-phosphoglycerate to phosphopyruvate.

**Phosphate Transfer from Phosphopyruvate to Adenosine Diphosphate**—Phosphopyruvate is broken down during glycolysis by transfer of its phosphate group to adenylic acid or adenosine diphosphate, to form pyruvate and adenosine triphosphate (25, 26) (Equation 4). This reaction must be studied in the absence of the enzyme adenosinetriphosphatase,

$$\text{Phosphopyruvate} + \text{adenosine diphosphate} + \text{H}_2\text{O} \rightarrow \text{pyruvate} + \text{adenosine triphosphate} + \text{OH}^-$$

which would hydrolyze the adenosine triphosphate formed. 3-Phosphoglycerate must also be absent; otherwise the adenosine triphosphate would break down by transfer of its phosphate to 3-phosphoglycerate to form 1,3-diphosphoglyceric acid, which spontaneously decomposes to 3-phosphoglycerate and inorganic phosphate (27). An acetone powder of an aqueous rabbit muscle extract (21) was used as the source of the enzyme in studying the transfer of phosphate from phosphopyruvate to adenosine diphosphate; such a powder does not contain an active adenosinetriphosphatase. Fluoride was added to prevent the formation of 3-phosphoglycerate from the phosphopyruvate (23, 28). Samples contained 0.025 M veronal buffer at pH 8.2, 0.005 M MgSO₄, 0.06 M KCl (29), 0.01 M NaF, 0.005 M phosphopyruvate, 0.0045 M adenosine diphosphate, and dialyzed extract equivalent to 10 mg. of acetone powder, in a total volume of 1.0 cc. In 10 minutes at 37° about 100 γ of phosphopyruvate P per cc. were transferred.

Quinine in concentrations from 0.0005 to 0.002 M caused no inhibition of this phosphate transfer.

**Phosphate Transfer from 3-Phosphoglycerate to Creatine**—Muscle extract catalyzes the transfer of phosphate from adenosine triphosphate to creatine (26) (Equation 5). The effect of quinine on the over-all process of phosphate transfer from 3-phosphoglycerate to creatine (the sum of Equations 3 to 5) was investigated. The reaction mixture consisted of 0.025 M veronal buffer at pH 8.7, 0.005 M MgSO₄, 0.06 M KCl, 0.03 M creatine, 0.00035 M adenosine triphosphate, 0.016 M 3-phosphoglycerate, and dialyzed extract equivalent to 5 mg. of acetone powder of rabbit muscle extract (21), in a total volume of 1.0 cc. The samples were incubated for 15 minutes at 37°. The reaction was followed by determining the increase in phosphocrea-
tine phosphate by the calcium precipitation method of Fiske and Subbarow (30). Balance experiments showed that the increase in phosphocreatine and inorganic phosphate accounted for 94 per cent of the 3-phosphoglycerate phosphate which disappeared, the rest being completely accounted for in phosphopyruvate and adenosine triphosphate. During the experimental period 240 γ of phosphocreatine P and 40 γ of inorganic P per cc. of reaction mixture were formed.

Quinine in concentrations from 0.0005 to 0.002 M caused no inhibition of the transfer of phosphate from 3-phosphoglycerate to creatine.

**DISCUSSION**

Silverman et al. (2) have found that the aerobic and anaerobic glycolysis of chicken erythrocytes parasitized with *Plasmodium gallinaceum* is inhibited about 35 per cent by 0.001 M quinine; i.e., by concentrations of the drug equal to those used in the studies reported in this paper. It would appear from our data that the effects described by Silverman et al. are in part due to the action of the drugs on the hexokinase and lactic dehydrogenase of the parasite, since these enzymes catalyze essential steps in the glycolytic process. The inhibition by quinine of the over-all process of lactic acid formation from glycogen by rabbit muscle enzymes is due to the effect of quinine on the enzyme phosphorylase. Likewise, the inhibition of the fermentation of glucose by intact yeast cells observed by Enders and Wieninger (31) may be ascribed to the inhibition of yeast hexokinase by the antimalarial.

In connection with the latter enzyme it should be pointed out that the hexokinase from parasitized red blood cells appears to be less sensitive to quinine than does the hexokinase of yeast and normal chicken erythrocytes, although the effect of atabrine on the enzymes from the three different sources is the same. We have not, as yet, any explanation for this difference in behavior. The other glycolytic enzymes from yeast and muscle show the same sensitivity to quinine and atabrine as the corresponding enzymes of the malaria parasites.

It should be emphasized that the concentrations of antimalarials needed to produce the effects described in this paper are considerably greater than those encountered in the blood and tissue fluids of animals being treated with the drugs. In recent studies on the *in vitro* distribution of quinine between parasitized chicken erythrocytes and a suspending medium, carried out by Dr. Joseph Ceithaml in this laboratory, it has been found that the ratio of intracellular to extracellular quinine concentrations is not greater than 50, even under circumstances which greatly favor the accumulation of quinine in the red blood cells. The concentration of quinine

2 Ceithaml, J. J., and Evans, E. A., Jr., unpublished work.
observed in the red cells of chickens receiving therapeutic doses of the drug is about $10^{-5}$ M (32). Therefore, unless there occurs a localization of the drugs inside the parasite cell, capable of producing high concentrations of the antimalarials in the immediate vicinity of the sensitive enzymes, it seems unlikely that inhibition of the glycolytic mechanism of the parasite is primarily involved in the therapeutic action of the drugs.

At present it seems more likely that quinine and atabrine inhibit oxidative processes in the metabolism of the malaria parasite, particularly those concerned with the oxidation of lactic and pyruvic acids. The oxygen consumption of the parasite utilizing glucose is inhibited by quinine in concentrations as low as $10^{-5}$ M (3). With these concentrations there occurs an accumulation of lactic acid, although the rate of glucose utilization is not affected (2). Therefore, it would appear that the effect of the drug is to inhibit the oxidative removal of lactic acid rather than its formation by the process of glycolysis.

**SUMMARY**

The effects of quinine and atabrine on some of the glycolytic enzymes of the malaria parasite (*Plasmodium gallinaceum*), yeast, and mammalian muscle were studied.

Atabrine inhibited the hexokinase activity and the enzyme lactic dehydrogenase in parasite preparations. Quinine was less effective in both cases. The enzyme 3-phosphoglyceraldehyde dehydrogenase was not affected by either drug.

Both quinine and atabrine inhibited yeast hexokinase.

Quinine inhibited the enzymes phosphorylase and phosphoglucomutase from rabbit muscle. Lactic dehydrogenase from beef heart was strongly inhibited by atabrine but only slightly affected by quinine. The other glycolytic enzymes from rabbit muscle which were studied were not affected by quinine.

The possible significance of these effects in the action of antimalarial drugs is discussed.

**BIBLIOGRAPHY**

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