ARGinine AND GLUCOSE METABOLISM IN A STRAIN OF STREPTOCOCCUS PYOGENES

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McIlwain (Biochem J. (London), 40, 67, 1946) reported that glutamine, arginine, and urea stimulate glycolysis in resting cells of the hemolytic streptococcus. We have observed an analogous phenomenon in cell-free preparations of a single strain of Streptococcus pyogenes (S23). Our cell-free extracts were prepared by grinding the washed cells from 1,600 ml of an 18 hour broth culture of a nonmucoid variant of strain

**TABLE 1**

_Glycolysis and arginine cleavage by cell-free extracts of Streptococcus pyogenes*

<table>
<thead>
<tr>
<th>Substrate</th>
<th>CO₂</th>
<th>NH₃</th>
<th>Lactic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µM/hr</td>
<td>µM/hr</td>
<td>µM/hr</td>
</tr>
<tr>
<td>Glucose</td>
<td>0</td>
<td>0</td>
<td>0.09</td>
</tr>
<tr>
<td>Glucose; ATP</td>
<td>0</td>
<td>0</td>
<td>0.20</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.7</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td>Arginine; AMP-5</td>
<td>7.9</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>Glucose; arginine</td>
<td>5.5</td>
<td>55</td>
<td>0.50</td>
</tr>
<tr>
<td>Glucose; arginine; ATP</td>
<td>8.3</td>
<td>35</td>
<td>0.72</td>
</tr>
<tr>
<td>Glucose; arginine; AMP-5</td>
<td>24.8</td>
<td>111</td>
<td>1.43</td>
</tr>
</tbody>
</table>

* Average results of multiple experiments with 13 cell-free preparations, corrected for endogenous activity.

Total volume: 3.0 ml; glucose: 30 mg/ml; arginine: 9 mg/ml; ATP: 3 mg/ml; AMP-5: 3 mg/ml; phosphate buffer: 0.15 M, pH 5.9.

S23 with 4 g of glass beads of 0.1 mm average diameter in 10 ml of distilled water for 15 minutes in a Mickle disintegrator (Mickle, J. Roy. Microscop. Soc., 68, 10, 1948). The extracts were freed of whole cells by high-speed centrifugation. A second nonenzymatic extract was obtained by boiling the sedimented cells for two minutes in 5 ml of distilled water. It was found necessary to add this boiled extract to the unheated extracts to obtain satisfactory enzymatic activity. All reactions were carried out at 37°C and at pH 5.9, except where otherwise indicated, using conventional Warburg techniques. Ammonia was determined by nesslerization after aeration from alkaline solution into saturated boric acid. Lactic acid was estimated by the method of Barker and Summerson (J. Biol. Chem., 138, 535, 1941).

Results of a study on the relationship between the glycolytic system and the arginine dihydrolase system studied by Slade and Slamp (J. Bacteriol., 64, 455, 1952) are given in table 1. While the extracts without supplementation were only feebly glycolytic, with added adenosine triphosphate (ATP) increased fermentation occurred, suggesting that adenosine triphosphatase (ATPase) might be active in the unboiled extracts. Slade (Arch. Biochem. and Biophys., 42, 204, 1953) has reported the probable presence of this enzyme in his streptococcal preparations.

With arginine as substrate there was rapid ammonia formation but very little carbon dioxide evolution, indicating that reaction 1 of the arginine dihydrolase system (arginine + H₂O → citrulline + NH₃) was proceeding while reaction 2 (citrulline + H₂O → ornithine + NH₃ + CO₂) was not. The addition of glucose or AMP-5 enhanced both carbon dioxide evolution and ammonia formation, and led to an increased carbon dioxide/ammonia ratio. Their combined effect was greater than additive. Conversely, arginine stimulated glycolysis.

We suggest the following hypothesis which may, in part, explain these findings: extract ATPase hydrolyzes ATP to ADP or AMP, so that glucose cannot be phosphorylated. However, if arginine is also present, its conversion to ornithine yields sufficient ATP (Slade, Doughty, and Slamp, Arch. Biochem. and Biophys., 48, 338, 1954) to initiate glycolysis, which in turn contributes still more ATP to the system. ATP is hydrolyzed by ATPase to ADP or AMP, each

1 This investigation was supported in part by a research grant from the National Microbiological Institute of the National Institutes of Health, U. S. Public Health Service.
of which has been shown to accelerate the conversion of citrulline to ornithine (Slade, Arch. Biochem. and Biophys., 42, 204, 1953).

Studies using phosphate buffers indicated that carbon dioxide formation occurred optimally in the range pH 5.9–6.1 in the glucose-arginine mixture, which is in agreement with Slade's observation (Arch. Biochem. and Biophys., 42, 204, 1953). Lactic acid production was also maximal in this range.

A CATALASE NEGATIVE MICROCOCCUS PYOGENES VAR. AUREUS

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Dr. T. F. Judefind of the College of Medical Evangelists at Loma Linda, California, isolated a gram positive, coagulase positive coccus from normal male urine with characteristics typical of Micrococcus pyogenes var. aureus with the unusual exception that catalase activity could not be found. The culture was sent to this laboratory for examination and was examined in the laboratory of Dr. James B. Evans at the American Meat Institute Foundation in Chicago. The original identification of the organism as a catalase negative Micrococcus pyogenes var. aureus has been verified.

The culture gives the following reactions: Acid is produced from glucose, lactose, maltose, sucrose, mannitol (aerobically and anaerobically) and glycerol, but not from xylose, fructose, raffinose, or inulin; nitrates are reduced to nitrites; arginine, esculin, and sodium hippurate are hydrolyzed whereas starch is not; gelatin is liquefied; an acid curd is produced in litmus milk; the coagulase test is strongly positive; no hemolysis in horse-blood agar occurs; on 1 per cent glucose agar a moist, smooth, yellowish growth is produced; hydrogen peroxide cannot be detected in aerated beef infusion broth cultures although growth is good; catalase activity cannot be shown by adding 3 per cent H₂O₂ to colonies on solid media of low glucose content. In manometric experiments, heavy cell suspensions and cell extracts prepared by sonic treatment failed to release oxygen from hydrogen peroxide.

The requirements of this culture for vitamins coincide with those found by Evans (J. Bacteriol., 55, 793, 1948) in his study of coagulase positive staphylococci, i.e., only nicotinic acid and thiamin are required.

Although the catalase test used commonly to distinguish streptococci from staphylococci has proven of great value, it is apparent that on rare occasions naturally occurring coagulase positive, catalase negative staphylococci may be encountered.

USE OF A DEHYDRATED CULTURE MEDIUM FOR SIMPLIFIED PREPARATION OF STREPTOCOCCAL CULTURES FOR GRIFFITH'S SLIDE AGGLUTINATION

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Serological typing of strains of Group A beta hemolytic streptococci by Griffith's slide agglutination technique (Griffith, J. Hyg., 34, 542, 1934) has been hampered by time consuming preparation of media for growth of test cultures and the tendency of cultures often to agglutinate spontaneously in these media. Media generally used contain fresh preparations of animal tissues, as in beef heart-trypsin digest broth (Pauli and Coburn, J. Exptl. Med., 65, 595, 1937) or modi-