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The assimilation and metabolism of guanidine by a strain of *Pseudomonas aeruginosa*

Although it is known that certain bacteria can desimidate substituted guanidines[1–6] and a creatinine desimidase has been partially purified[7], there has been no report on the bacterial metabolism of guanidine.

A strain of *Pseudomonas aeruginosa* was grown in 100 ml of Bacto Nutrient Broth for 24 h at 35°. After washing in water by repeated centrifugation the cells were suspended in 10 ml 0.05 *M* Na-K-phosphate, pH 7.6, and 0.5 ml of this used in a final volume of 2.0 ml. After incubation at 37°, 0.2 ml 20 % trichloroacetic was added and after centrifugation aliquots of the supernatant were taken for the estimation of guanidine by the method of WEBER[8] using a 490 mμ filter. Methylguanidine was also estimated by this method and equivalent amounts gave 85 % of the color produced by guanidine.

When 100 μg guanidine·HCl were added to the washed cells 10–15 μg disappeared in 15 min. After that no further disappearance was evident unless an oxidizable substrate such as pyruvate was added. 30–60 min after the addition of pyruvate the rest of the guanidine began to disappear and continued to completion. This is shown in Fig. 1. This disappearance, *i.e.* assimilation, of guanidine depended on the presence of an oxidizable substrate because assimilation ceased when oxidation ceased. Thus, within limits, the amount of guanidine assimilated should be proportional to the amount of substrate added. Fig. 2 shows that this is so for pyruvate in the range of 1.0–2.5 mg. The assimilation of NH_4^+ is also dependent on oxidation and NH_4^+ completely inhibited guanidine assimilation until no extracellular NH_4^+ remained.

Following assimilation, guanidine was metabolized since none was found in the cells after they were broken by sonic vibration. The most likely mechanism is desimidation to form NH_4^+ and urea followed by the breakdown of urea by urease. Urease

can be induced in these cells by the addition of pyruvate[9]. (Endogenous purines are metabolized to urea in the presence of an oxidizable substrate.) Thus, if urea is formed from guanidine, the presence of previously induced urease should increase the rate of guanidine assimilation. Fig. 3 shows that a 60-min pre-incubation of the cells with pyruvate before the addition of guanidine does this. Pre-incubation with pyruvate has no effect on NH_4^+ assimilation.

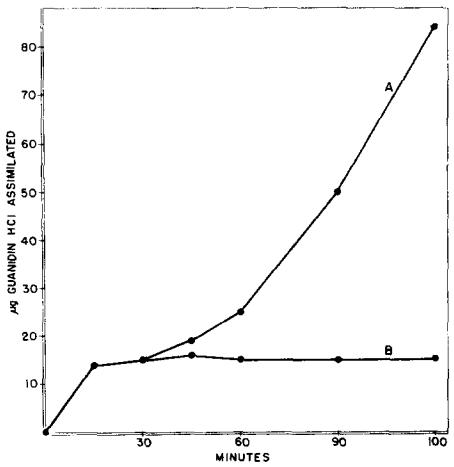

Fig. 1.

Fig. 1. The assimilation of 100 μg guanidine·HCl in the presence (A) and absence (B) of 2.0 mg Na pyruvate.

Fig. 2. The assimilation of 100 μg guanidine·HCl after 240 min incubation in the presence of differents amounts of Na pyruvate.

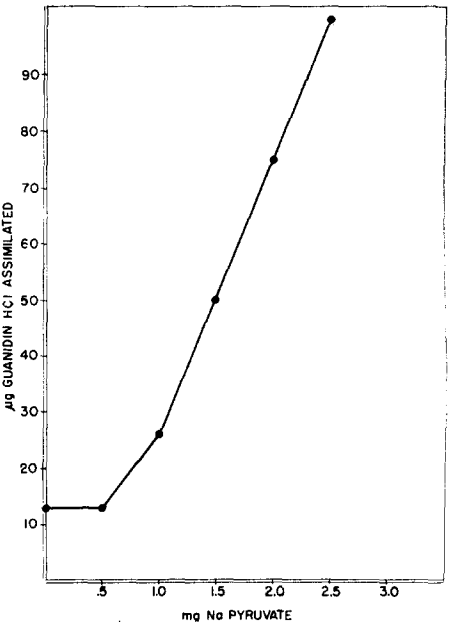

Fig. 2.

It was possible to show that all the nitrogen in guanidine was available for utilization by the cell. This was done by inducing the enzymes for the oxidation of benzoate and comparing the effect of guanidine with that of an equivalent amount of nitrogen added as $(NH_4)_2SO_4$. Shortly after the oxidation of benzoate began, energy was available for the assimilation of the NH_4^+, and the apparent oxidation rate of benzoate and its products was increased. This is shown in Fig. 4 (non-nitrogenous compounds in equimolar amounts were without effect on the oxidation rate). The curves on the left represent the increase in oxidation rate in the presence of 4.2 and 8.4 μg $(NH_4)_2SO_4$ over that of the control. When guanidine was substituted for $(NH_4)_2SO_4$ the increase began later and proceeded at a slow rate at first. With urea the increase began still later. But once urease was induced, the oxidation rate in the presence of both guanidine and urea increased rapidly. It is possible to infer from this that the imino nitrogen of guanidine became available first, followed by the other two nitrogen atoms once urease was induced. The extent of the increase in oxidation rate was roughly proportional to the amount of nitrogen present as shown for $(NH_4)_2SO_4$ in Fig. 4. The proportionality was also true for the other compounds. The increase in rate ceased only because the nitrogen was limiting.

It seems probable that the latent period in the assimilation of guanidine repre-

sents the time necessary for the induction of a desimidase. Two facts indicate that this is so. (a), Chlortetracycline (0.05 μg/ml), which inhibits enzyme induction in this organism, completely inhibited guanidine assimilation when added at the beginning but caused only partial inhibition when added during the linear phase of the reaction.

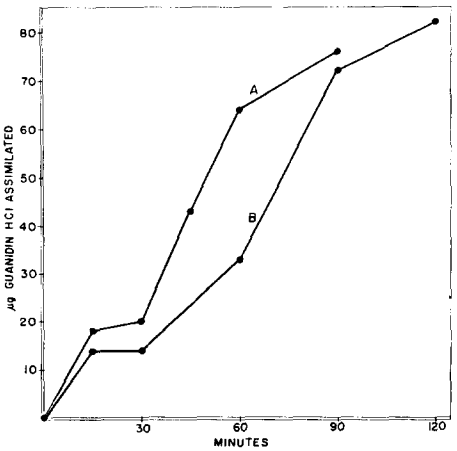

Fig. 3. The effect of 60 min pre-incubation with pyruvate on the assimilation of 100 μg guanidine·HCl. A, pre-incubated. B, control.

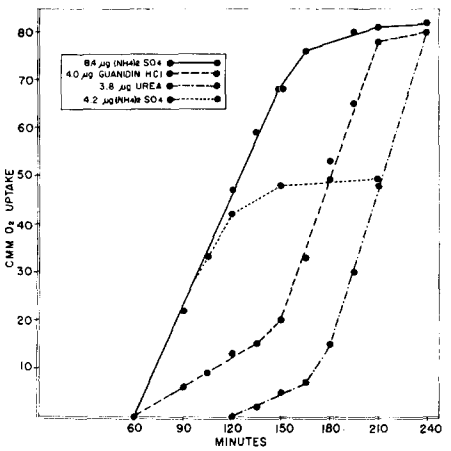

Fig. 4. The increase in oxidation rate of 0.5 mg Na benzoate in the presence of various nitrogenous compounds. The oxygen uptake of benzoate alone has been subtracted.

(b) Increasing the nitrogen pool by allowing the cells to assimilate NH_4^+ before the addition of guanidine increased the rate of guanidine assimilation (NH_4^+ completely inhibits urease induction).

All the above facts apply to methylguanidine except that there was a longer latent period before assimilation began. Since all three of its nitrogen atoms were available to the cell and since urease does not hydrolyze methylurea, methylguanidine was probably demethylated. Creatinine was assimilated after a much longer latent period and apparently only one of its nitrogen atoms became available. There was no evidence that glycocyamine was metabolized.

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