NOTES

Effect of Hydroxyurea on *Staphylococcus epidermidis* and *Micrococcus lysodeikticus*: Thickening of the Cell Wall

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Hydroxyurea-sensitive strains of *Staphylococcus epidermidis* and *Micrococcus lysodeikticus* showed marked thickening of cell walls and reduction in deoxyribonucleic acid synthesis when grown in the presence of hydroxyurea.

Although hydroxyurea (HU) is primarily an inhibitor of deoxyribonucleic acid (DNA) synthesis, in a previous report (3) we described a strain (FR) of *Staphylococcus epidermidis* which recovered spontaneously from the effects of HU. In its presence, DNA synthesis was reduced, but not inhibited. Autolysis of washed, exponentially growing cells was inhibited when they had been previously exposed to HU (2). Ultrastructural studies revealed no striking differences between normal and HU-treated cells (unpublished data).

We have now isolated an isogenic HU-sensitive mutant strain, FR-1, from *S. epidermidis* strain FR by treatment with *N*-methyl-*N*¹-nitro-*N*-nitrosoguanidine (1). The mutant, like the parent strain, is sensitive to chloramphenicol (30 μ g) and streptomycin (10 μ g) by the disk method and is resistant to penicillin (125 IU/ml) by the tube dilution method. The procedures used to characterize the mutant have been described (3).

The mutant, after a lag phase of about 2 h, grew at a slower rate than the parent strain and was much more sensitive to HU (0.1 M final concentration) (Fig. 1; reference 3). Incorporation of ³H-thymidine into DNA was decreased after a delay of 1 h by approximately 50% (Table 1). This lack of immediate inhibition of DNA synthesis was also seen in the HU-resistant parent strain (3). The mutant, however, was intrinsically autolytic defective. During the latter part of exponential growth, untreated FR-1 showed a reduction in turbidity of only 15% as compared with 92% for the parent strain (Table 2; 2) when tested under the same conditions. Autolysis in FR-1 was not significantly inhibited further by

FIG. 1. Growth of *S. epidermidis* (FR-1) in the presence of 0.1 M hydroxyurea (HU). Cultures were brought to the log phase of growth, and HU was added at this time ($T = 0$). The number of viable bacteria was determined at intervals; ●, control; ○, HU-treated. Turbidities (at 450 nm) of undiluted samples are shown in parentheses.

previous exposure of the cells to HU (0.1 M) either in the growth medium or after growth in HU-free medium (Table 2). Autolysis of FR cells was inhibited under these conditions.

Electron microscopic examination revealed a significant increase in the thickness of the cell wall after growth of *S. epidermidis* FR-1 in the

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FIG. 2. Untreated *S. epidermidis* FR-1 after 24 h of growth. $\times 116,600$.

FIG. 3. *S. epidermidis* FR-1 after 24 h of growth in the presence of 0.1 M hydroxyurea. $\times 116,600$.

FIG. 4. Growth of *M. lysodeikticus* (ATCC 4698) in the presence of 0.1 M hydroxyurea (HU). Cultures were brought to the log phase of growth, and HU was added at this time ($T = 0$). The number of viable bacteria was determined at intervals; ●, control; ○, HU-treated. Turbidities (at 450 nm) of samples diluted 10-fold are shown in parentheses.

presence of HU (Fig. 2 and 3). (Cultures were prepared for electron microscopy as follows: bacteria were fixed for 2 h at room temperature

TABLE 1. Incorporation of ³H-thymidine in the presence of hydroxyurea^a

Organism	Intervals after addition of ³ H-thymidine (h)	Radioactivity (counts per min per ml)	
		Control	HU-treated
<i>S. epidermidis</i> (FR-1)	1	682	662
	2	1,163	628
	3	1,652	766
	4	2,363	993
<i>M. lysodeikticus</i> (ATCC 4698)	1	910	713
	2	1,343	802
	3	1,885	1,008
	4	2,464	1,158

^a Cultures were prepared as described in Fig. 1. Hydroxyurea (HU; 0.1 M) and ³H-thymidine were added at onset of log phase.

in 1.5% glutaraldehyde (cacodylate-buffered at pH 6.8). After washing, the specimens were post-fixed in 1% osmium tetroxide overnight at 4 C, stained in 2% uranyl acetate, dehydrated in ethyl alcohol, and embedded in epoxy resin (Epon 812). Sections were stained with uranyl

FIG. 5. Untreated *M. lysodeikticus* (ATCC 4698) after 19 h of growth. $\times 40,500$.

FIG. 6. *M. lysodeikticus* (ATCC 4698) after 19 h of growth in the presence of 0.1 M hydroxyurea. $\times 40,500$.

TABLE 2. Autolysis of *S. epidermidis*^a

Strain	Treatment	Age of culture from onset of log phase (h)	Reduction in turbidity after 20 to 22 h of incubation at 37 C
FR	None	2.5	92
FR-1	None	4.0	15
FR-1	HU-treated in broth culture	4.0	9
FR-1	HU-treated in buffer	4.0	12

^a Cultures were grown as described in Fig. 1. Untreated and hydroxyurea (HU)-treated (0.1 M) cells were washed and suspended in tris-(hydroxymethyl)aminomethane - citrate - NaCl buffer (2) prior to incubation for autolysis. Turbidity was measured at 450 nm.

acetate followed by lead hydroxide and were examined in a Phillips 200 electron microscope.)

Micrococcus lysodeikticus strain ATCC 4698 was also sensitive to HU. This organism was sensitive to chloramphenicol (30 μ g) and streptomycin (10 μ g) by the disk method and very sensitive to penicillin (2 IU/ml) by the tube dilution method. Characteristically, it was a slow grower with a lag period of about 4 to 5 h in broth media. Like *S. epidermidis* FR-1, but unlike the FR strain, *M. lysodeikticus* did not recover colony formation when grown in the presence of HU (Fig. 4). Judging from incorporation of ³H-thymidine, DNA synthesis likewise was not immediately inhibited (Table 1). Although no observations on the autolysis of washed cells were made at this time, in our experience with this organism, we have not observed spontaneous lysis of young broth cultures, a phenomenon not infrequently observed with staphylococci. There was no question, however, that in this case also

treatment with HU resulted in marked thickening of the cell wall (Fig. 5 and 6).

In the HU-sensitive cocci described above, the most marked effect which appears after prolonged exposure to HU is thickening of the cell wall. Nevertheless, that this may be an indirect effect following initial and continuing effects elsewhere in the cell cannot be discounted, because DNA synthesis is eventually reduced. As has already been pointed out (3), the unbalanced growth, here manifested morphologically in the cell wall, may reflect a series of reciprocal events following a primary attack either on the cell wall or the membrane or possibly DNA itself. In any case, because HU blocks autolysis in a HU-resistant staphylococcus and produces cell wall thickening in a HU-sensitive, autolytic-defective mutant, it may be interfering with regulatory mechanisms involving autolytic enzymes which are indispensable in normal, cell wall development (4).

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