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Effect of Hematin on the Recovery of *Bacillus anthracis* and Related Organisms

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Van Heyningen (Nature **162**:114, 1948) reported that growth of *Bacillus anthracis* was not inhibited on nutrient agar containing 50 μg of hematin per ml, although certain other spore-bearing aerobes were inhibited. Pearce and Powell (J. Gen. Microbiol. **5**:387, 1951) developed a selective medium for *B. anthracis* containing hematin and lysozyme. Preliminary work in this laboratory on the development of a selective medium for *B. anthracis* indicated that quantitative recovery was dependent on the age of hematin solutions added to the medium. Freshly prepared alkaline hematin solutions were found to be inhibitory for gram-positive organisms.

Hematin solutions were prepared by dissolving 400 μg of hemin (Eastman Kodak Co., Rochester, N.Y.) per ml of 0.01 N NaOH and autoclaving for 30 min at 5 psi. (Hemin changes to hematin in the presence of alkali.) A stock hematin solution was stored at 4 C and used periodically in test media. This permitted the determination of the [illegible]nal aging time required for full recovery of [illegible]s test organisms. The test medium was prepared by adding either freshly prepared or aged hematin solutions to Heart Infusion (HI) Agar (Difco) at a final concentration of 40 μg/ml (*p*H 7.4); the medium was used after overnight preincubation (37 C). The effect of *p*H was evaluated by adjusting the media in a range of *p*H 6.6 to 8.2. The test inoculum was prepared by removing growth from a 24-hr HI Agar slant and suspending it in 0.067 M phosphate buffer. Dilutions of the suspension were adjusted to contain 10^3 organisms per milliliter, and 0.1 ml was added to each plate (triplicate plates used and incubated at 37 C).

Recovery of various organisms on the test media is shown in Table 1. All of the *Bacillus* species, *Staphylococcus aureus*, and three *Streptococcus* strains were markedly inhibited in the presence of fresh hematin.

The inhibition of soil organisms was significantly increased, but *Escherichia coli*, *Aerobacter aerogenes*, *Pseudomonas aeruginosa*, and four strains of *Pasteurella pestis* were not inhibited by freshly prepared hematin solutions. Further inhibition of gram-positive organisms occurred when the concentration of fresh hematin was increased, but full recovery was obtained on media containing as much as 80 μg/ml of aged hematin (2 months at 4 C). Recovery of some strains on media containing aged hematin was more than double the recovery on the HI Agar control. Fresh hematin solutions prepared with

TABLE 1. *Per cent recovery of various organisms on media containing fresh and aged hematin*[a]

Test organism[b]	No. of strains	Avg recovery[c]	
		Aged hematin[d]	Fresh hematin[e]
		%	%
Bacillus anthracis	10	95–163	0–20
B. anthracis (spore suspension)	1	90	0
B. cereus	9	83–266	0–23
B. cereus var. *mycoides*	4	78–240	0–4
B. circulans	1	222	10
B. lentimorbus	1	100	8
B. megaterium	4	93–111	0–23
B. polymyxa	1	238	1
B. pumilus	1	100	0
B. sphaericus	2	94–96	16–18
B. sphaericus var. *funiformis*	1	80	1
B. subtilis	5	60–133	0–53
B. thuringiensis	2	122–147	0–2
Staphylococcus aureus	1	111	3
Streptococcus faecalis	4	59–133	0–115
Aerobacter aerogenes	1	97	92
Escherichia coli	1	78	85
Pseudomonas aeruginosa	1	95	103
Pasteurella pestis	4	106–141	94–126

[a] The amount of hematin was 40 μg/ml.

[b] Growth from 24-hr HI agar slant, except spore suspension which was stored in distilled water at 4 C.

[c] Heart Infusion Agar as 100% recovery control.

[d] Alkaline hematin solution stored at 4 C for 2 months.

[e] Medium containing fresh hematin was used within 24 hr after preparation.

hematin from two different sources (Nutritional Biochemicals Corp., Cleveland, Ohio, and Eastman Kodak Co.) were equally inhibitory. A 14-day aging period of hematin was required for full recovery of *B. cereus* and *B. subtilis*; 3 to 4 weeks were required for *B. cereus* var. *mycoides*, *S. aureus*, and *B. anthracis*. Similar results were obtained when nutrient agar was used in place of HI Agar. Changing the *p*H of the medium containing fresh hematin had no significant effect on recovery of test organisms. The composition of the alkaline hematin solution when freshly prepared, and the changes that occurred during storage, were not determined.

Kammerer (Verhandl. Deut. Kongr. Inn. Med. **31**:704, 1914) reported that many gram-positive organisms (including *B. anthracis*) were inhibited by mesohemin, whereas gram-negative organisms were generally not inhibited. He was also able to achieve complete inhibition of *B. anthracis* and *B. megaterium* with a 1:300 dilution (3,333 μg/ml) of hematin (age not specified).

Marked variation in the recovery of *B. anthracis* and many other gram-positive organisms will occur on media containing hematin if the age of the hematin used is not specified. This variation will also occur when fresh hematin and lysozyme are combined, as in the medium of Pearce and Powell (with a peptone agar base).

In summary, the results indicate that freshly prepared alkaline hematin solution is inhibitory for many gram-positive organisms, including *B. anthracis;* therefore, its use in a selective medium for the isolation of *B. anthracis* is questionable. However, freshly prepared alkaline hematin has been found useful in a selective medium for *Pasteurella pestis* (Knisely, Swaney, and Friedlander, J. Bacteriol. **88**:491, 1964). Further work on a selective medium for *B. anthracis* is in progress.

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