Inhibition of Pigment Formation of *Serratia marcescens* by Chloramphenicol, Aureomycin, and Terramycin. (19438)

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The production of red pigment ("prodigiosin") by *Serratia marcescens* is subject to spontaneous variation(1,2), which presumably is genetically controlled. Moreover, pigment formation depends on certain external factors, such as temperature and the supply of magnesium and iron(3,4). Glutamic acid has just been identified by Weinberg (5) as an inhibitor of pigment production. A similar effect of chloramphenicol (chloromycetin), aureomycin, and terramycin is here described.

Methods. Four strains of prodigiosus bacilli were collected in this hospital during the summer of 1951. The fifth strain used was originally obtained from Dr. E. C. Roberts of St. Louis(7,8). The organisms were grown on 2% nutrient agar (Baltimore Biological Laboratories), pH 7,3. Temperature is not critical between 20°C and 37°C. Commercially prepared disks (Difco) were employed according to the method recently described(6) for preliminary tests with chloromycetin, aureomycin, terramycin, penicillin, streptomycin, di-hydrostreptomycin, and bacitracin. More quantitative tests were done on agar slants containing weighed amounts of antibiotic* covering a wide range of concentrations in 2-fold steps. The slants were inoculated immediately after preparation, except in some experiments, where duplicates were left at room temperature (Approximately 72°F) for 2 days in order to obtain information on the stability of the antibiotics in nutrient agar.

Results. No inhibition of coloration or of growth was seen around disks containing penicillin, streptomycin, di-hydrostreptomycin, and bacitracin. In fact, a narrow zone of enhanced pigmentation was on occasion seen around disks with penicillin or bacitracin, which is in keeping with Weinberg's findings (5). However, wide zones of colorless growth were shown around disks with chloromycetin. The same was true for aureomycin and terramycin, though zones were somewhat narrower in width than those around chloromycetin.

TABLE I. Inhibitory Effect of Chloramphenicol, Aureomycin, and Terramycin on Growth and Pigment Formation of *S. marcescens*.

Antibiotic	Conc. necessary to inhibit: Growth, μg/ml	Conc. necessary to inhibit: Pigment formation, μg/ml
Chloramphenicol	640-1280*	10-20
Aureomycin	160- 640	10-40
Terramycin	±5000*	5-20

* One exception (see text).

The results of experiments on slants, into which antibiotics were incorporated, are summarized in Table I. Inhibition of growth by chloromycetin was obtained at concentrations between 640 and 1280 μg/ml, with the exception of one strain, which was inhibited by 40 μg. Pigment formation comparable to that of controls growing on nutrient agar without drug appeared at levels of 10 or 20 μg. With aureomycin, inhibition of growth was found to vary between 160 and 640 μg/ml, and pigment formation returned at levels between 10 and 40 μg. Terramycin inhibited growth only at the highest level tested, which was 5000 μg/ml (assuming complete solution of the amount in the agar, of which it was difficult to be sure). The only exception was the strain which had been found relatively susceptible to chloromycetin also, and which was inhibited by 160 μg/ml. Pigment formation in the presence of terramycin reappeared at levels between 5 and 20 μg. With all 3 agents, there was a trace of pink coloration visible in one or 2 tubes above the levels quoted for full pigmentation. These data refer to readings made after a period of in-

* Chloromycetin, aureomycin, and terramycin were provided by the courtesy of Parke, Davis & Co., of Lederle Laboratories Division, American Cyanamid Co., and of Chas. Pfizer & Co., respectively.

cubation of about 18 hours. After 2 or more days, little change was seen with chloramphenicol. With terramycin, pigmentation increased in one or 2 tubes above the levels given in the preceding paragraph. With aureomycin, both partial growth and some pigmentation appeared in 2 to 4 tubes above the levels quoted. This could be correlated with the stability of the antibiotics. If growth and pigment formation on media, which had been kept for 2 days after addition of the antibiotic, was compared with that of media inoculated immediately after preparation, no noticeable difference was seen in the case of chloromycetin. With terramycin, about 50% of the inhibiting effect had disappeared. The nutrient agar containing aureomycin showed a loss of activity of over 90%.

If the colorless population from slants with antibiotic was plated out on nutrient agar, the ratio of red to white colonies was not significantly different from that resulting from platings of the same strain grown without drug. It appears therefore, that—excepting rare resistant mutants—pigment formation is suppressed only as long as the antibiotic is present. Also, no significant competitive advantage is gained either by the pigmented nor the colorless variant under the conditions of our experiments.

On 4 occasions, one or 2 red colonies appeared among the colorless growth at concentrations of antibiotic 2 to 8 times that generally inhibiting pigmentation. In 3 unrelated observations a few isolated colonies were noted growing well above the levels of general inhibition of growth. In these cases, growth was colorless and remained so upon propagation on media free of antibiotic. It appears that 2 kinds of mutants occurred, one showing resistance to the growth inhibiting effect of antibiotics, and one able to produce pigment in the presence of concentrations of antibiotic which usually suppress this function.

Weinberg(5) reported that penicillin and bacitracin antagonize the inhibiting effect of glutamic acid upon pigment formation. In search for similar effects, plates with discs of chloromycetin, aureomycin, and terramycin, each in concentrations of 60, 30, and 10 μg, were set up, where discs with 10 units of penicillin or 20 units of bacitracin were placed either immediately adjacent to or 7 mm distant from the first named discs. No influence upon the zones of colorless growth was noticed under these conditions.

It may be mentioned that 3 strains of heavily pigmented strains of *Pseudomonas aeruginosa* and one strain of a pink yeast were tested by the disc method against the 7 antibiotics enumerated above. No inhibition of pigment formation nor, for that matter, of growth, were observed.

These data may perhaps open an avenue of approach to the problem of the mode of action of chloramphenicol, terramycin, and aureomycin. In this connection it is of some interest that prodigiosin is a tripyrrylmethane, and as such related to the respiratory pigments (9,10).

Summary. Chloramphenicol, aureomycin, and terramycin inhibit the production of pigment by *Serratia marcescens* over a wide range of concentrations, at which the growth of the organism is not inhibited.

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