MODE OF ACTION OF CHLORAMPHENICOL VIII.

RESEMBLANCE BETWEEN LABILE CHLORAMPHENICOL-RNA AND DNA OF BACILLUS CEREUS

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Received December 26, 1961

When chloramphenicol is removed from bacterial cultures that have been exposed to this antibiotic for several hours, a considerable portion of the RNA that has accumulated intracellularly in the absence of protein synthesis is degraded and excreted into the recovery medium (Hahn et al., 1957). This degradation proceeds beyond the nucleotide stage, uracil and hypoxanthine having been identified as two of the RNA products (Neidhardt and Gros, 1957). We have found that guanine and adenine, as well as small quantities of their respective nucleosides and nucleotides are also excreted.

The present work was based upon the assumption that upon removal of the antibiotic from a bacterial culture chloramphenicol-RNA is degraded enzymatically, adenylic acid giving rise to a mixture of adenine and hypoxanthine and uridylic and cytidylic acids being converted to uracil. It was hoped that quantitative analysis of the post-chloramphenicol bacterial excretion products for purine and pyrimidine bases would produce data that might be indicative of the base composition of the unstable portion of the original chloramphenicol-RNA. Such analyses were performed on the RNA breakdown products excreted by Bacillus cereus after release from chloramphenicol action. Assuming the enzymatic transformations postulated above, it was found that the relative abundance of the RNA bases resembled that of the corresponding bases in the DNA of B. cereus rather than that of the overall RNA of this organism.

B. cereus was selected as the test organism because its DNA and RNA differ greatly with respect to the relative abundance of their corresponding constituent bases (Belozersky and Spirin, 1960). The test strain (kindly furnished by Dr. Falkow of the Department of Bacterial Immunology of this Institute) was grown in brain-heart infusion broth (Difco) until the cultures had attained an optical density of 0.25 at 420 mμ, as measured in a Beckman DU spectrophotometer. Chloramphenicol (50 μg per ml) was then added, and the incubation continued for 90 minutes. The bacteria were harvested and washed twice with distilled water at 4°C. Finally, the collected organisms were resuspended in isotonic phosphate-buffered NaCl solution at pH 7.3 to 1/20 of the volume of the original culture. These suspensions were incubated for 180 minutes, aliquots being withdrawn at intervals, filtered through Millipore filters and subjected to spectrophotometry at 260 mμ. Fig. 1 depicts the time course of the release of 260 mμ-absorbing material in a typical experiment. Eventually the entire suspension was filtered through Millipore filters, and aliquots of the filtrates were subjected to paper chromatography, employing a butanol-formic acid-water solvent (Markham and Smith, 1949), and to column chromatography on Dowex 50 (Heinrich et al., 1952).

Uracil, guanine, adenine, and hypoxanthine were identified by their chromatographic behavior and by their optical properties. Neither cytosine nor thymine were detected. Small quantities of UV-absorbing material remained on the starting position of paper chromatograms, suggesting the presence of nucleotides. Hydrolysis of the filtrates in 1 N HCl for 1 hour at 100°C prior to chromatography eliminated these appearances. In such hydrolysates the four bases mentioned above were the only UV-absorbing substances detected. Column chromatography and spectrophotometric quantitation produced the analytical data listed in Table 1. Typical DNA and RNA compositions for B. cereus are tabulated for comparison.

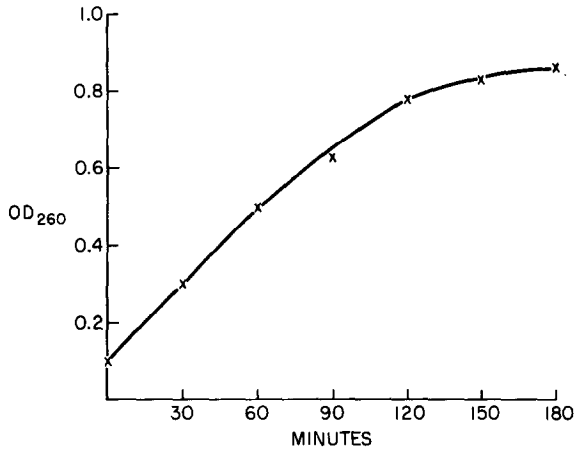

Figure 1.—Release of UV-absorbing Material from B. cereus during Recovery from Chloramphenicol Action.

TABLE I

Base Composition of Nucleic Acids of B. cereus in Mole Per Cent

DNA*				
Guanine	Adenine	Cytosine	Thymine	Cytosine+Thymine
17.8	32.2	17.5	32.6	50.1
Chloramphenicol-RNA Fragments				
Guanine	Adenine+ Hypoxanthine	-	-	Uracil
20.8±0.9	29.8±0.5	-	-	49.3±0.6
RNA*				
Guanine	Adenine	Cytosine	Uracil	Cytosine+Uracil
31.0	25.5	20.1	23.4	43.5

*The mole per cent data for DNA and RNA are mean values calculated from the tables of Belozerski and Spirin (1960).

When adenine-8-C^{14} was added to chloramphenicol-exposed test cultures and excess label removed along with chloramphenicol by washing as described above, the subsequent excretion of 260 mμ-absorbing material was paralleled by an excretion of radioactivity from the bacilli. All the excreted radioactivity was recovered as adenine and hypoxanthine, and both bases exhibited

identical specific radioactivities. Therefore we consider these findings as evidence that hypoxanthine was derived from the adenine moiety of chloramphenicol-RNA.

Finally, it was found that cell-free extracts of alumina-ground B. cereus contain an active cytidine deaminase (Wang et al., 1950). We believe that the absence of cytosine and the presence of high concentrations of uracil among the RNA-degradation products of the bacilli were the result of the action of this enzyme in the dissimilation of chloramphenicol-RNA.

Instability, and a base composition patterned after that of the DNA of the respective cell of origin, are considered to be characteristics of messenger RNA, postulated to be the template material in protein synthesis (Gros et al., 1961). When their protein synthesis is inhibited by chloramphenicol, bacteria produce RNA which is unstable* and is degraded into a set of bases whose pattern of relative abundance in the present study resembled the base composition of the DNA of the test organism.

We are entertaining the hypothesis that chloramphenicol inhibits protein synthesis by interfering with the function of messenger RNA, i.e., of the template. In an earlier publication (Hahn et al., 1956) the action of chloramphenicol as a "template poison" has been discussed from the viewpoint of the chemical structure of the antibiotic. While we do not wish to speculate at this time on the various possible mechanisms by which chloramphenicol could interfere with the function of messenger RNA, such a mechanism must allow for the accumulation of this RNA in chloramphenicol-exposed bacteria. Further studies on this problem are under way and will be communicated.

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*According to Horowitz et al. (1958), RNA also exhibits its instability while it is being formed in the presence of chloramphenicol.

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