STIMULATION OF STAPHYLOCOCCAL NITRATE REDUCTASE PRODUCTION BY CHLORAMPHENICOL*

H. H. Ramsey

Department of Medical Microbiology, Stanford University, Stanford, California and Research Corporation, 1290 Bayshore Highway, Burlingame

Received January 28, 1966

That chloramphenicol is a powerful inhibitor of protein synthesis has been confirmed numerous times since the initial observation of Gale and Folkes (1953). Further, the antibiotic has been found by Sypherd, Strauss, and Treffers (1963) to preferentially inhibit the formation of inducible enzymes at concentrations which permit exponential growth and synthesis of constitutive enzymes by Escherichia coli. Therefore, it was surprising to find in repeated experiments in this laboratory that Staphylococcus aureus synthesized nitrate reductase more rapidly in the presence of chloramphenicol than in its absence. Simultaneously, it was noted that chloramphenicol caused 90% inhibition of C^{14}-arginine incorporation into the protein fraction. (Table 1)

*This investigation was supported by a grant from the Research Corporation.

Table 1

Nitrate reductase formation and incorporation of C^{14}-arginine into protein by S. aureus

Time, hr.	CM, μg/ml.	Nitrite produced (M x 10^{-5})	C^{14}-Arginine incorporation
1	0	2.0	2.55#
	50	6.4	1.42
2	0	4.8	5.82
	50	16.8	1.69
3	0	20.3	10.36
	50	81.6	1.39

#Radioactivity (in mμc/ml.) of the cellular fraction insoluble in hot 5% trichloroacetic acid. Specific activity of C^{14}-arginine was 24.8. Each ml. of cell suspension contained 100 μg protein per ml., as determined by the method of Eagle and Oyama (1956).

In the experiment shown, a 14 hr. culture was washed and resuspended, in Thunberg tubes to a final volume of 8 ml. in a synthetic growth medium containing KNO_3 (1%) and U-C^{14}-arginine (10 μc/ml.). The tubes were evacuated and at the time intervals noted, 2 ml. samples were removed. The cells were washed with phosphate buffer (pH 7.2) and resuspended to 2 ml. One ml. was mixed with an equal volume of 10% trichloroacetic acid and placed in a boiling water bath for 10 min. After centrifuging, the radioactivity of the precipitate was determined by combustion to $C^{14}O_2$ and measurement of radioactivity on a Dynacon electrometer (Ramsey, 1962). For nitrate reductase determination, one ml. was suspended in 4 ml. of 50%

acetic acid. One ml. of Gries-Ilovay reagent was added and the cells centrifuged. The color produced was measured at 550 mμ and is an expression of the concentration of nitrite in the suspension (Pollock, 1946).

It may be seen that after 1 hr. incubation in the presence of 50 μg cloramphenicol/ml., over three times as much nitrate reductase was produced as in the absence of the antibiotic. Total protein synthesis, as measured by incorporation of labeled arginine, was inhibited approximately 45% under identical conditions. After 3 hr. incubation, nitrate reductase formation was enhanced almost 4 fold by chloramphenicol, whereas C^{14}-arginine incorporation was reduced by 88%. This experiment has been performed several times with comparable results. With this same strain of S. aureus, 10 μg CM/ml. has been found to completely inhibit growth.

In subsequent experiments it has been found that chloramphenicol alone does not serve as an inducer of nitrate reductase -- nitrate must be present for enzyme formation. Further, within the range of 5 - 50 μg chloramphenicol per ml., formation of enzyme is proportional to concentration. Under similar conditions, chloramphenicol does not induce the formation of chloramphenicol reductase (Smith and Worrel, 1949) or chloramphenicolase (Miyamura, 1964), nor does it give a positive color test with the Gries-Ilovay reagent used to measure production of nitrite from nitrate.

ACKNOWLEDGEMENT

The technical assistance of Judith Ogrodnik is acknowledged.

REFERENCES

Eagle, H. and Oyama, M., Proc.Soc.Exptl.Biol.Med., 91, 305(1956).
Gale, E. F. and Folkes, J., Biochem. J., 53, 493 (1953).
Miyamura, S., J. Pharm. Sci., 53, 604 (1964).
Pollock, M. R.,Brit. J. Exptl. Path., 29, 223 (1946).
Ramsey, H. H., J. Bacteriol., 83, 507 (1962).
Ramsey, H. H. and Wilson, T. E., Nature, 180, 761 (1957).
Smith, G. N. and Worrel, C. S., Arch. Biochem., 24, 216 (1949).
Sypherd, P. S., Strauss, N., and Treffers, H. P., Biochem. Biophys. Res. Communs., 7, 477 (1962).