NOTES

EFFECT OF SULFADIAZINE ON THE SYNTHESIS OF DEMETHYLCHLORTETRACYCLINE BY STREPTOMYCES AUREOFACIENS

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Demethylchlortetramycylne produced by a mutant strain of Streptomyces aureofaciens Duggar (McCormick et al., J. Am. Chem. Soc. 79:4561, 1957) differs from chlortetracycline only in the lack of a methyl group at carbon-6 (Webb et al., J. Am. Chem. Soc. 79:4563, 1957). This suggested that interference with 1-carbon transfer mechanisms might result in the formation of demethylchlortetramycylne by a strain which normally produces only chlortetracycline and small amounts of tetracycline. By use of a system previously described (Goodman et al., J. Bacteriol. 78:492, 1959), a number of sulfamidamide drugs, p-aminobenzoic acid analogues, and folic acid antagonists were added to the medium. Identification and semiquantitation of the products were made by paper chromatography.

Twenty out of forty sulfonamides tested resulted in formation of demethylchlortetracycline. The compounds, which were active when used at from 30 to 100 μg/ml, were all substituted benzene or substituted nitrogen heterocyclic derivatives. The most active included sulfapyridines, sulfadiazines, and sulfapyrazines, with methyl, methoxy, thiomyethyl, or chlorine substituents on the aromatic moiety. Diaminodihydrophenylsulfone was also somewhat active. All sulfonamides causing demethylchlortetramycylne formation also caused a marked reduction in growth and a reduction in potency from a level of 5,000 or 6,000 μg/ml to 500 μg/ml. However, the ratio of demethylchlortetramycylne to chlortetracycline in these broths ranged from 10 to 65%.

Sulfadiazine used at 50 and 70 μg/ml resulted in the formation of 30 and 65% demethylchlortetramycylne out of totals of 825 and 510 μg/ml, respectively. In further experiments, it was found that its effect both on growth and demethylchlortetramycylne synthesis could be readily reversed by p-aminobenzoic, folic, and folinic acids. This implies that p-aminobenzoic acid is transformed by the well-known cycle via folic and folinic acids to the coenzymes responsible for the introduction of 1-carbon units into various metabolites. In contrast to the sulfonamides, p-aminobenzoic acid analogues, such as p-aminosalicylic, p-nitrobenzoic, and 2-chloro-4-aminobenzoic acids (Wang et al., J. Biol. Chem. 188:753, 1951), did not result in the formation of demethylchlortetramycylne. Similarly ineffective were the folic acid antagonists, aminopterin, amethopterin, pyrimethamine (Hitchings, Clin. Pharmacol. and Therap. 1:570, 1960), and several diaminoxydhydrortiazines (Lux, Antibiotics & Chemotherapy 4:971, 1954). Pyrimethamine did, however, have a synergistic effect with sulfadiazine (Eyles and Coleman, Antibiotics & Chemotherapy 3:483, 1953).

The inhibitory effect of sulfadiazine on the growth of S. aureofaciens could be overcome by adding it 16 hr after the fermentation was initiated. Similarly, repeated transfer of the contents of a fermentation flask into media of increasingly higher sulfadiazine content resulted in a culture resistant to 500 μg/ml of the drug. In both cases where normal growth was achieved, no demethylchlortetramycylne was produced. Cultures made resistant to sulfadiazine were not examined further.

Since the strain used was capable of producing high levels of tetracycline, we attempted to promote the formation of demethylchlortetramycylne by using sulfadiazine in chloride-free media (Doerschuk et al., J. Am. Chem. Soc. 81:3069, 1959) or media containing chlorination inhibitors (Goodman et al., J. Bacteriol. 78:492, 1959). In these systems only tetracycline along with some chlortetramycylne was produced. The reason for this failure is at present obscure.

The introduction of a C6-methyl group into the basic tetracycline structure appears to be a folic and folinic acid-dependent reaction which can be blocked by sulfonamides. The marked growth inhibition evident when effective sulfonamides are used suggests that one or more other folic acid-dependent reactions are also blocked.