# SYNERGY AND CO-TRIMOXAZOLE SENSITIVITY

S.G.B. Amyes, Department of Bacteriology, The Medical School, University of Edinburgh, Teviot Place, Edinburgh EH8 9AG, United Kingdom

The agar diffusion technique is the most commonly used method to determine the sensitivity of bacteria to co-trimoxazole and in most cases a single sensitivity disk containing 1 part trimethoprim and 20 parts sulphamethoxazole is used. This procedure is contrary to the guidelines of the Expert Committee on Antibiotics of the World Health Organisation (1961) which stated that two drugs should never be tested using a single disk. Therefore an alternative to the single disk using two separated disks was investigated.

Trimethoprim was marketed in conjunction with sulphamethoxazole to exploit the synergy between the two drugs and it seemed prudent to test for this as well as drug sensitivity to the individual drugs. Synergy has been shown between disks containing the two drugs by a bridging of the zones of sensitivity in a standard agar diffusion sensitivity test (Waterworth, 1969). However, this has always been a qualitative observation and a number of parameters affect the result. The optimum ratio for synergy is one part trimethoprim to 20 parts sulphamethoxazole. This is an absolute ratio related to the minimum inhibitory concentration of both drugs. Most laboratory media, however, antagonize sulphonamides to a greater extent than trimethoprim and thus reduce the effective sulphonamide concentration. To compensate for this the content of the sulphonamide disk may have to be raised. Therefore sensitivity tests were performed with one disk of 1μg trimethoprim and another of increasing concentrations of sulphamethoxazole. A disk containing 50μg sulphamethoxazole gave the best demonstration of synergy and this took into account the degree of sulphonamide antagonism.

The sensitivity tests were repeated using separate disks of 1μg trimethoprim and 50μg sulphamethoxazole at different spacings. With both sensitive and resistant organisms a distance of 25mm between the centres of the two disks was the optimum to demonstrate synergy. This spacing was crucial for when the distance was greater the disks were too far apart to influence one another and if it was less the zones of sensitivity overlapped. The choice of laboratory media is important in testing the sensitivity of bacteria to the components of co-trimoxazole. When a variety of laboratory media were used to test for synergy it was found that they affected the demonstration of synergy to a lesser extent than the sensitivity of the individual drugs. The media that were most suitable for testing sensitivity to these drugs (Amyes & Smith, 1974; 1976) also gave the clearest demonstrations of synergy. The inoculum of bacterium may affect the result of sensitivity tests and so, to determine whether it has an effect on the demonstration of synergy, the sensitivity test was performed using various dilutions of an overnight culture of the sensitive strain Escherichia coli NCTC 10418. As the dilution increased, synergy was more readily seen. However, at dilutions in excess of 1 in 10,000 there was no further improvement.

A trial was performed testing overnight cultures of clinical isolates, diluted 1 in $10^4$, with one disk of trimethoprim (1μg) and one of sulphamethoxazole (50μg) spaced at 25mm. The medium used was Wellcotest Sensitivity Test Agar. There was an exact correlation between the demonstration of synergy using this method and the more laborious technique of plotting the fractional inhibitory concentrations on an isobologram (Elion, Singer and Hitchings, 1954).

Amyes, S.G.B. & Smith, J.T. (1974). J. Med. Microbiol., 7, 143-153.
Amyes, S.G.B. & Smith, J.T. (1976). J. Pharm. Pharmac., 28, Suppl., 52P.
Elion, G.B., Singer, S. & Hitchings, G.H. (1954). J. Biol. Chem., 208, 477-488.
Second Report of the Expert Committee on Antibiotics (1961). Geneva W.H.O. Technical Report Series, no. 210.
Waterworth, P.M. (1969). Postgrad. Med. J., 45, Suppl. , 21-27.