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## The Future of Trimethoprim

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From a dissertation read before the Society on 11th February, 1981

### Abstract

Co-trimoxazole became available for medical use towards the end of 1968 and represented a successful marriage of the old and the new, being a combination of a sulphonamide and a new drug called trimethoprim. Today it is one of the most widely prescribed drugs in the United Kingdom, and is used extensively in urinary and respiratory tract infections. More recently, however, trimethoprim has been released for use on its own, and this has raised considerable controversy as to which is the drug of choice.

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# THE FUTURE OF TRIMETHOPRIM

AW McKinlay, BSc (Hons)

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Co-trimoxazole became available for medical use towards the end of 1968 and represented a successful marriage of the old and the new, being a combination of a sulphonamide and a new drug called trimethoprim. Today it is one of the most widely prescribed drugs in the United Kingdom, and is used extensively in urinary and respiratory tract infections. More recently, however, trimethoprim has been released for use on its own, and this has raised considerable controversy as to which is the drug of choice.

## Three original claims for co-trimoxazole

Simple laboratory tests suggest that a combination of trimethoprim and a sulphonamide will inhibit bacterial growth at concentrations lower than either drug on its own. More formal assays which compare the drugs over a range of concentrations confirm that the antibacterial effect of the combination greatly exceeds a purely additive response. The drugs' interaction is said to be synergistic, although no single definition of the term synergy has ever been universally accepted.

- (1) The first claim made for co-trimoxazole was that this synergistic effect would prove beneficial in the clinical situation<sup>1</sup>.

Two major categories of antibacterial drug have been described. Bacterio-static drugs merely prevent the multiplication of a bacterial culture, but the organisms remain viable and will grow again if transferred to a fresh medium. Bactericidal drugs actually kill the bacteria, and the number of viable organisms in the culture declines.

- (2) The second claim put forward for co-trimoxazole was that although trimethoprim and sulphamethoxazole are bacteriostatic on their own, their combination is bactericidal.

- (3) Finally, it was believed that a combination of trimethoprim and sulphamethoxazole would

prevent the emergence of trimethoprim-resistant bacteria.

Fundamental to this last claim was the work of Darrell, Garrod and Waterworth (1968)<sup>2</sup> which is still widely quoted. Large inocula of bacteria were exposed to increasing concentrations of trimethoprim, and resistant strains were shown to emerge. Resistance could not be induced so readily when organisms were exposed to a combination of trimethoprim and sulphamethoxazole.

## Bacterial folate metabolism

Biochemical pathways involving the transfer of single carbon units utilise a co-factor to which the carbon moiety is temporarily attached. In the majority of cases the co-factor is tetrahydrofolic acid (THF). Important cellular processes that require THF include the synthesis of a number of amino-acids and the formation of purines which are amongst the basic building blocks of DNA and RNA. Of particular interest is the synthesis of thymidine from deoxyribose uridine monophosphate (dUMP). In this complex reaction THF not only acts as a methyl donor but also as a reducing agent, being oxidised to dihydrofolate (DHF) in the process. The cell reconverts DHF to THF via the enzyme dihydrofolate reductase (DHFR) which is crucial to the maintenance of the cellular pool of THF. Many of the reactions involving THF are broadly similar in both mammalian cells and in bacteria, but they differ greatly with respect to their source of new folate. Mammals absorb preformed, exogenous folate from dietary sources whilst most bacteria must synthesise it *de novo* from dihydropteridine and para-aminobenzoic acid (PABA) (Fig. 1). Often bacteria can absorb exogenous PABA but few can absorb folate itself.

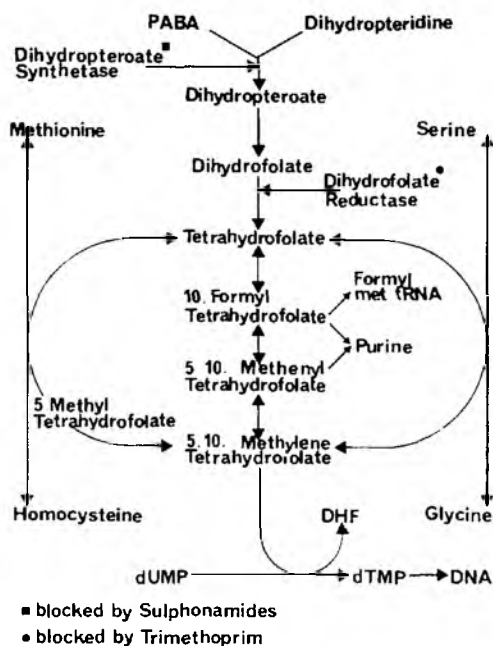


Fig. 1: The synthesis of folic acid and some important reactions using THF.

### The metabolic actions of sulphonamides and trimethoprim

Woods (1941)<sup>3</sup> showed that the antibacterial properties of sulphonamides could be antagonised by PABA, and it is now accepted that sulphonamides act as PABA analogues and competitively inhibit the dihydropteroate synthetase (DHPS) (Fig. 1). Since mammalian cells do not possess this enzyme only bacteria become folate depleted. Moreover, since most bacteria cannot take up folate they are unable to bypass the blockade. After the Second World War research started on antimetabolites of folic acid itself resulting in a number of small molecular inhibitors, including trimethoprim, which exploit subtle differences between the DHFRs found in different species.

Sulphonamides and trimethoprim, therefore, sequentially blockade the synthesis of THF by the bacterial cell, and this is the conventional explanation for their synergistic interaction.

This argument has come under increasing attack recently: firstly it assumes that the folate pathway is a linear one, when in fact it is not.

The production of thymidine at the expense of THF and its subsequent reversion to DHF introduces a cyclical series of reactions. Trimethoprim inhibits the crucial step and so halts the cycle. On purely theoretical grounds, therefore, it would seem that trimethoprim is probably the more important member of the partnership. Opponents of sequential blockade argue that the degree of inhibition is entirely dependent on the more effective of the two inhibitors, which is trimethoprim. An analogy can be drawn to a water-pipe on which there are a number of taps. The flow rate in the pipe is dictated by whichever tap is shut off the most. Other taps on the pipe make no difference to the overall flow rate. It has also been shown that some bacteria entirely resistant to sulphonamide show a classic synergistic effect when this is combined with trimethoprim. The DHPS in such strains is known to be insensitive to sulphonamide and it is extremely difficult to explain the synergistic effect using a sequential blockade model.

Poe (1976)<sup>4</sup> has produced evidence which suggests that sulphonamides may bind not only to the DHPS but also to the bacterial DHFR. It is suggested that binding the sulphonamide somehow improves the activity of trimethoprim. In support of this are reports that sulphonamides will inhibit haematopoiesis in mouse bone marrow, which does not contain a DHPS. The effect is reversed by folate and it appears that the sulphonamide is inhibiting the DHFR.

### Bacterial resistance to sulphonamides and trimethoprim

Chromosomal resistance to both trimethoprim and sulphonamides is known to occur. The DHPS or DHFR may gradually mutate to a form less sensitive to inhibition by the drugs. Alternatively, the cell may produce vast quantities of enzyme and in this way maintains a pool of active enzyme molecules. Some sulphonamide-resistant staphylococci overproduce PABA and so displace the sulphonamide from the DHPS. These chromosomal mechanisms rarely produce high level resistance. I suspect that these were the mechanisms that the experiments of Darrell, Garrod and Waterworth (1968)<sup>2</sup> selected (see page 1.).

Two chromosomal mechanisms confer total

resistance to the cell. The first is an impermeability mechanism and is usually found in species that are intrinsically resistant. Secondly, some bacterial strains have dispensed with the enzyme thymidine synthetase, and so avoid degrading their pools of THF. Growth is maintained by the absorption of exogenous thymidine. Despite their somewhat perilous life-style, thymidine-dependent mutants can be pathogenic in man and are sometimes isolated from urinary tract infections.

A second type of bacterial resistance is carried on "plasmids" – small circular pieces of DNA, distinct from the chromosome, that can replicate and maintain themselves stably over many generations. It has been known for many years that some plasmids can transfer themselves from one bacterial cell to another. It has been found more recently that some resistance genes can jump from one plasmid to another, a process known as "transposition".

Plasmid mediated resistance has been found to both sulphonamides and trimethoprim. Two mechanisms for sulphonamide resistance occur. Firstly, the production of a new DHPS insensitive to sulphonamide, and secondly an impermeability mechanism.

Only one major mechanism for trimethoprim resistance has been shown, and this involves the production of a highly insensitive DHFR. This enzyme is, unfortunately, highly specific for DHF, and will not bind trimethoprim or methotrexate (which resembles folic acid very closely). The prospects of designing a new inhibitor to block the enzyme are therefore remote.

#### In-vitro studies on co-trimoxazole

Recently the original claims for co-trimoxazole have been reassessed by a number of workers, and I am very grateful to Dr. S. Amyes (Department of Microbiology, University of Edinburgh) for allowing me to quote his recent work on this subject.

Fig. 2 shows a culture of *Escherichia coli* grown in a minimal medium, that is a medium containing glucose and some inorganic salts. Trimethoprim and sulphonamide are bacteriostatic and the growth of the culture is prevented. The original claim forecast that their combination should be bactericidal, but under these conditions the combination is only bacteriostatic. This tends to refute the second claim.

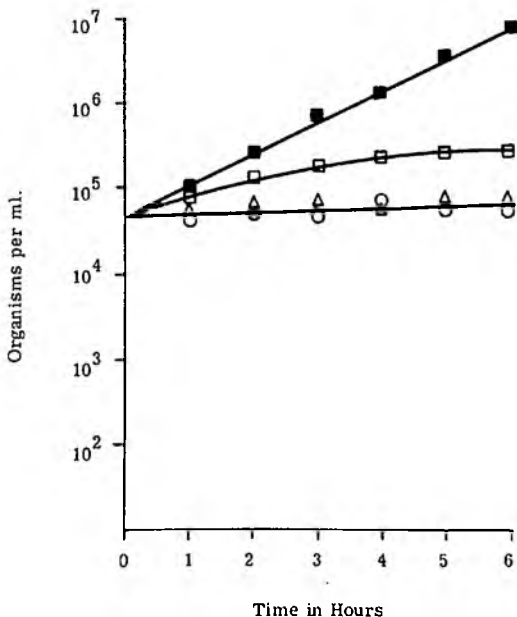


Fig. 2: Effect of trimethoprim and sulphamethoxazole on the viability of *E. coli* K<sub>12</sub> in DM. An exponential culture was diluted into pre-warmed medium containing no additions ■ : trimethoprim (5 mg l<sup>-1</sup>) △ ; sulphamethoxazole (100 mg l<sup>-1</sup>) □ ; trimethoprim (5 mg l<sup>-1</sup>) and sulphamethoxazole (100 mg l<sup>-1</sup>) O.

Co-trimoxazole is widely used to treat respiratory and urinary tract infections. Conditions in the respiratory tract are difficult to model *in vitro* but specimens of urine are much easier to obtain and to analyse. Amyes and Smith (1974)<sup>5</sup> were able to show that conditions in the urine could be duplicated quite closely by a minimal medium with supplements of some amino acids. The addition of methionine, glycine and serine produces conditions that duplicate urine as far as trimethoprim and sulphamethoxazole action is concerned.

When the previous experiment is repeated using this medium it can be shown that 0.04 µg/ml trimethoprim has no effect on growth on its own, while 0.1 µg/ml is bacteriostatic (Fig. 3a) Above 0.1 µg/ml, however, trimethoprim becomes markedly bactericidal when used on its own. The concentration of trimethoprim achieved in the urine varies between 100-200

$\mu\text{g/ml}$ . Sulphamethoxazole (Fig. 3b) never becomes bactericidal even at concentrations of  $100 \mu\text{g/ml}$ .

In summary, therefore, under conditions similar to those found in the urine co-trimoxazole is bactericidal whereas sulphonamide is only bacteriostatic. At concentrations greater than  $0.1 \mu\text{g/ml}$  trimethoprim becomes markedly bactericidal by itself. The second claim put forward for the combination is, therefore, not supported by this experiment.

Does synergy occur under conditions similar

to those found in the urine? Fig. 4 shows the effect of trimethoprim and sulphamethoxazole combined in various ratios.  $0.04 \mu\text{g/ml}$  trimethoprim on its own has no antibacterial action, but as the concentration of sulphonamide is increased the combination becomes increasingly active, reaching its maximum effect at a trimethoprim: sulphamethoxazole ratio of 1:20 that is  $0.04 \mu\text{g/ml}$  of trimethoprim to  $0.8 \mu\text{g/ml}$  sulphamethoxazole. Synergy, therefore, can occur under conditions similar to those found in the urinary tract. Unfortunately the concentration of trimethoprim

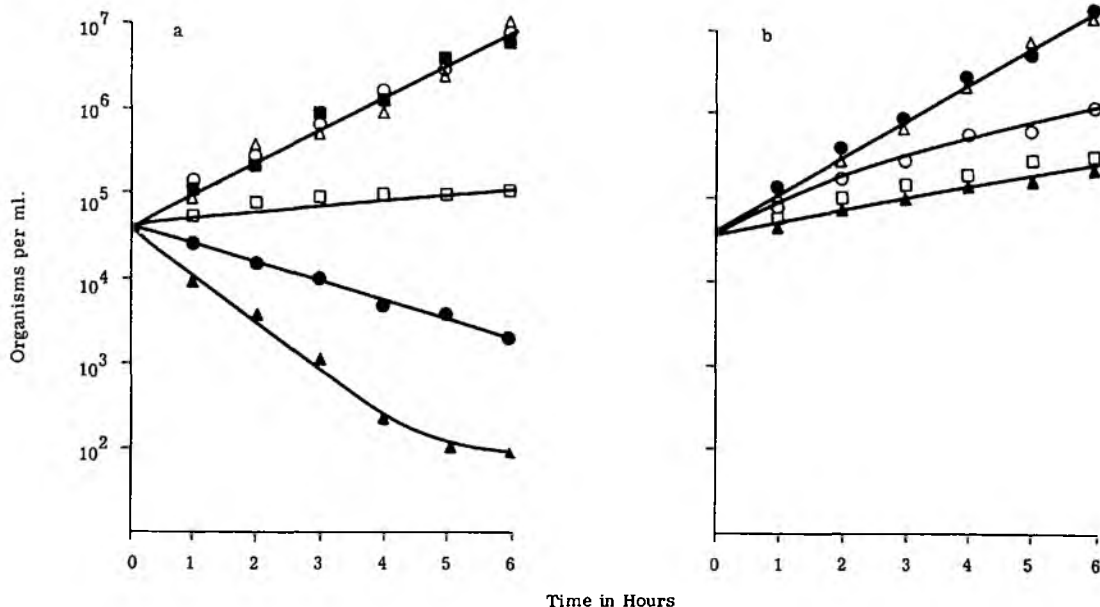


Fig. 3 a & b: Effect of trimethoprim and sulphamethoxazole on the viability of *E. coli* K<sub>12</sub> in DM supplemented with methionine, glycine and adenine ( $50 \text{ mg l}^{-1}$  each). An exponential culture was diluted into pre-warmed medium containing the following anti-microbial

drugs. In (a) trimethoprim was used at  $0 \text{ mg l}^{-1}$   $\blacksquare$ ;  $0.02 \text{ mg l}^{-1}$   $\circ$ ;  $0.04 \text{ mg l}^{-1}$   $\triangle$ ;  $0.1 \text{ mg l}^{-1}$   $\square$ ;  $0.2 \text{ mg l}^{-1}$   $\bullet$ ;  $0.4 \text{ mg l}^{-1}$   $\blacktriangle$ ; In (b); sulphamethoxazole was used at  $0 \text{ mg l}^{-1}$   $\bullet$ ;  $0.4 \text{ mg l}^{-1}$   $\triangle$ ;  $0.8 \text{ mg l}^{-1}$   $\circ$ ;  $2.0 \text{ mg l}^{-1}$   $\square$ ;  $100 \text{ mg l}^{-1}$   $\blacktriangle$ .

achieved in the urine is not  $0.04 \mu\text{g/ml}$  but  $100 \mu\text{g/ml}$ , at which it is markedly bactericidal on its own. Moreover, the trimethoprim: sulphamethoxazole ratio is not 1:20 but 1:2 and there is no useful synergistic effect. Synergy would not appear, therefore, to be of any practical value in the urinary tract. Similar arguments apply to the respiratory tract. Indeed there are only two areas in the body where the drugs are present at the correct ratio for synergy, and they are the aqueous humour of the eye and synovial fluid. Synergy is unlikely to be of value except in these rather specialised areas.

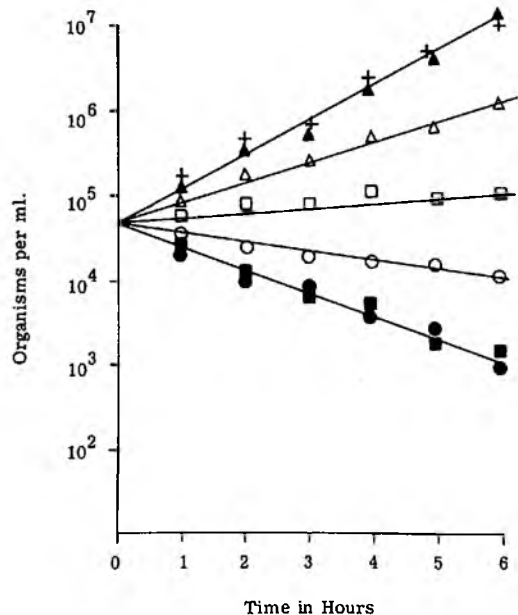


Fig. 4: Effect of trimethoprim and various concentrations of sulphamethoxazole on the viability of *E. coli* K<sub>12</sub> in supplemented DM. An exponential culture was diluted into pre-warmed medium containing trimethoprim at  $0.04 \text{ mg l}^{-1}$  and sulphamethoxazole at 0,  $0.04 \text{ mg l}^{-1}$  ▲;  $0.08 \text{ mg l}^{-1}$  △;  $0.2 \text{ mg l}^{-1}$  □;  $0.4 \text{ mg l}^{-1}$  ○;  $0.8 \text{ mg l}^{-1}$  ●;  $4.0 \text{ mg l}^{-1}$  ■.

So far I have considered the action of the drugs on sensitive organisms. Figs. 5a and b show two strains carrying plasmids that confer resistance to sulphonamides. Plasmid SSu confers

an impermeability type mechanism and no synergy occurs because the sulphonamide cannot enter the cell. R1 codes for an insensitive DHPS, and yet synergy occurs with trimethoprim. This suggests that sulphonamide may also bind to the DHFR and in this way potentiates the action of trimethoprim. It is of no practical value, however, because the cell is sensitive to trimethoprim and would therefore be destroyed whether the sulphonamide was present or not.

Figs. 6a and b show two strains carrying plasmids that confer resistance to trimethoprim. R483 confers high level trimethoprim resistance, that is to concentrations  $> 1000 \mu\text{g/ml}$ . The addition of sulphonamide produces no demonstrable synergy.

R751 also confers high level ( $> 1000 \mu\text{g/ml}$ ) resistance to trimethoprim, but in this case the addition of sulphonamide produces a striking enhancement of trimethoprim activity. The combination is markedly bactericidal and far better than either drug on its own. This is a remarkable result and represents an area where synergy would clearly be of advantage.

How do R483 and R751 differ? Studies on the enzymes produced by each plasmid show them to be similar, possibly even the same enzyme, when compared using simple biochemical criteria. The major difference appears to be the quantity of enzyme produced by each plasmid. A cell containing R483 produces more enzyme than R751, although quite how this renders it less susceptible to synergy is difficult to see.

Unfortunately this is of little clinical relevance because the majority of plasmid mediated resistance genes found in clinical isolates are of the R483 type and are therefore totally resistant to trimethoprim, irrespective of whether the sulphonamide is present or not. R751 resistance is very rare having been found once in London, and possibly once in Edinburgh. It is therefore of little concern clinically.

The claim that synergy occurs and is of value in the clinical situation is not supported by these experiments. Other *in vitro* studies have also pointed to the dominant role of trimethoprim.

#### Clinical findings on co-trimoxazole

The third claim made for co-trimoxazole was that it would delay the emergence of trimethoprim-

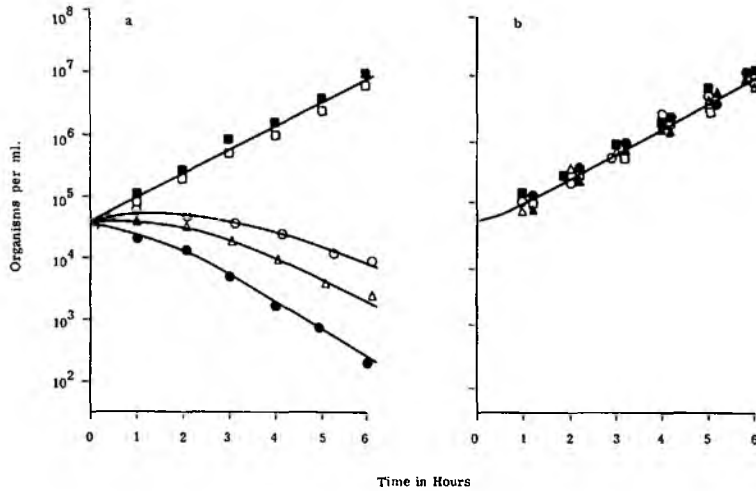


Fig. 5 a & b: Effect of trimethoprim and sulphamethoxazole together on the viability of *E. coli* K<sub>12</sub> containing the sulphamethoxazole-resistant R-plasmids (a) R1 and (b) SSu. Exponential cultures were diluted into pre-warmed

medium containing trimethoprim at 0.04 mg l<sup>-1</sup> ■ or sulphamethoxazole at 100 mg l<sup>-1</sup> □; and trimethoprim at 0.04 mg l<sup>-1</sup> plus sulphamethoxazole at 0.8 mg l<sup>-1</sup> ○; 1.6 mg l<sup>-1</sup> △; 3.2 mg l<sup>-1</sup> ● 100 mg l<sup>-1</sup> ▲ .

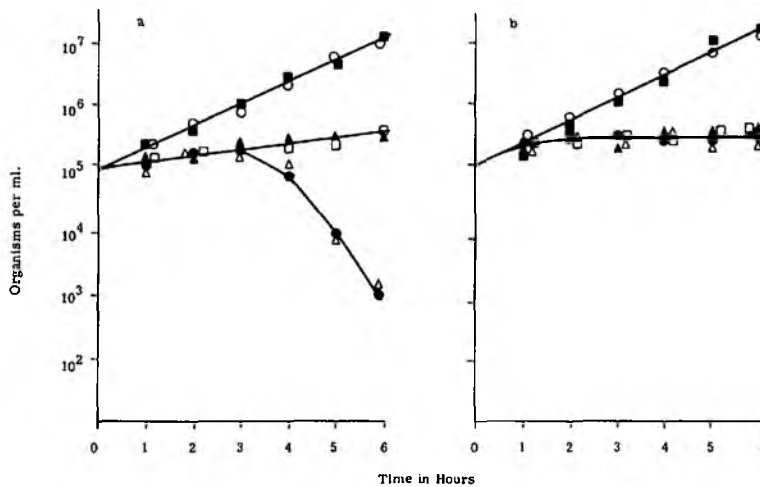


Fig. 6 a & b: Effect of trimethoprim sulphamethoxazole together on the viability of *E. coli* K<sub>12</sub> containing the trimethoprim-resistance plasmids (a) R751 and (b) R483. Exponential cultures were diluted into pre-warmed medium con-

taining trimethoprim at 5 mg l<sup>-1</sup> ■ or 50 mg l<sup>-1</sup> ○; sulphamethoxazole at 100 mg l<sup>-1</sup> ▲ or 1000 mg l<sup>-1</sup> □; trimethoprim/sulphamethoxazole together at 5 mg l<sup>-1</sup>/100 mg l<sup>-1</sup> ● or 50 mg l<sup>-1</sup>/1000 mg l<sup>-1</sup> △ .

resistant bacteria. Sulphonamide resistance was common amongst bacteria even in 1968 when co-trimoxazole was introduced. It is difficult to put a precise figure on any resistance level because there is tremendous variation between surveys carried out in different areas. This probably reflects differences in the samples chosen for study; e.g. inpatients vs outpatients, the range of organisms encountered, local antibiotic usage patterns, and possibly laboratory test methods. Many studies suggest, however, that sulphonamide resistance is present in over 50% of all clinical bacteria, so many must have already been exposed to trimethoprim alone.

Resistance to trimethoprim was found very

soon after its introduction, R-plasmids being isolated within one year of co-trimoxazole coming on to the market: few people suspected that R-plasmids would emerge this quickly.

It is difficult to estimate the current level of trimethoprim resistance. Work by Grüneberg (1980)<sup>6</sup> suggests that resistance amongst urinary tract pathogens has not increased dramatically since its introduction. Undoubtedly, more hospital acquired infections are resistant. Table 1 reviews a number of reports from European centres. French workers reported a high incidence of resistance (17%), whilst the incidence rose in Italy from 13% to 30% over a three-year period. Other continental reports show similar levels.

TABLE 1

Percentage of organisms with minimum inhibitory concentration of greater than 10 mg l<sup>-1</sup> trimethoprim and those possessing trimethoprim R-plasmids (shown in parentheses).

	PAVIA	PARIS	LONDON	LONDON	NOTTINGHAM	TURKU
	ITALY	FRANCE	U K			FINLAND
1973	13(1.6)	17(0)				
1974	25(1.9)	17(6.6)				
1975	30(7.1)		8(0.8)			
1977			10(1.4)	11(2.7)		
1978					5(0.6)	22(0)
1979					3(1.0)	

Reports from the United Kingdom initially showed low levels of 2.5% and 4.3% but this increased from 8% to 10% in London over the period 1977-1978. Taken as a whole these figures suggest a gradual increase in resistance, which makes the figures from Nottingham (which show a decline), rather surprising. Closer examination shows that there was a small epidemic of resistant *Kelbsiella* spp. in 1978, which probably biases the result, but the level of resistance must still be relatively low. A recent study carried out in a number of Edinburgh hospitals suggests a much higher level of resistance (26%) than has been found before.

Turku is an area in Finland where trimethoprim has been used extensively on its own. A figure of 20% resistance amongst isolates is high, but is certainly in keeping with other areas where only the combination has been used, such as Edinburgh. The results of these surveys, although limited, suggest that resistance in an area where trimethoprim has been used alone is probably no higher than in many places where only cotrimoxazole has been used. A number of reports have found lower resistance levels. This may reflect sampling isolates from general practice or from hospitals serving different populations, or from areas where the use of co-trimoxazole has been



more limited.

If the percentage of resistance that is plasmid-mediated is examined, then a trend is revealed. Undoubtedly the percentage of plasmid-mediated trimethoprim resistance is increasing (even in Nottingham), and the range of plasmids is probably diversifying as the trimethoprim resistance genes transpose on to new plasmids.

In summary, therefore, resistance to trimethoprim varies from centre to centre. It is always higher in hospitals and in areas where trimethoprim has been used extensively, irrespective of whether it is combined with a sulphonamide or not. Plasmids carrying trimethoprim resistance are becoming increasingly common, and those carrying resistance to sulphonamides have been widespread for many years. I see no suggestion that the sulphonamide has in any way prevented the emergence of trimethoprim resistance and this would suggest that the third claim made for the combination is not valid.

There are some similarities between trimethoprim resistance and ampicillin resistance. Ampicillin was released in 1960 and plasmids were found within one year. Initially most resistance was chromosomal but gradually the percentage due to R-plasmids increased (Richards and Datta 1981)<sup>7</sup> and the plasmid pool diversified as the TEM- $\beta$ -lactamase transposed on to new plasmids. In the early 1970's, ampicillin resistance spread to *Haemophilus influenzae* and then to *Neisseria gonorrhoeae*. It worries me that trimethoprim resistance is also capable of transposition and I wonder if R-plasmid trimethoprim resistant *Haemophilus spp.* may emerge one day.

### Conclusions

A hypothetical story relates that a group of aeronautical engineers once analysed the aerodynamic properties of the bumble-bee and "proved" that it couldn't fly. Co-trimoxazole to the bee; it doesn't work on paper but is "flies" in practice, and therefore why abandon a drug that obviously works well in the clinical setting?

I would suggest that the undoubted success of co-trimoxazole is due to trimethoprim, and to trimethoprim alone. Various clinical studies have shown no significant difference between co-trimoxazole and trimethoprim. Only one study, by Gleckman (1973)<sup>8</sup> has ever shown any

significant advantage for the combination in the clinical situation. In this study over one third of the organisms cultured were from species of *Klebsiella*, *Enterobacter* and *Proteus*, which can be very variable in their resistances. No attempt was made to find the source of the bacteriuria, which could therefore reflect anything from pyelonephritis to a simple bladder infection. Courses of treatment were carried on for up to 55 days. Finally, although the level of sulphonamide resistance was assessed, no assessment of trimethoprim resistance was carried out. I do not think that this trial can be accepted in view of this omission.

There may be disadvantages to using co-trimoxazole. Sulphonamides are notorious for their side-effects, including Stevens-Johnson syndrome which is a severe form of erythema multiforme. It is rare but carries a high mortality. Wellcome have suggested that adverse reactions to co-trimoxazole are seldom severe: rashes occur in about 1.6-8% of patients and gastrointestinal reactions such as nausea and vomiting are usually mild. Up to June 1976 five cases of fatal agranulocytosis due to Septrin had been reported to the Committee for the Safety of Medicines, which represents about one fatality in every 50 million treatment courses. Under-reporting of side-effects, particularly if mild, has always been a problem. For example, a surveillance study in the West Midlands was able to find 19 serious rashes, including three Stevens-Johnsons, one of which was fatal and one neutropenia/thrombocytopenia which was fatal. Co-trimoxazole is now the second most common cause of drug-induced thrombocytopenia in the United Kingdom, seven cases being reported in the West Midlands, and four cases of agranulocytosis — one of which was fatal. Two further cases were not reported. This regional survey confirms that the national figures underestimate the incidence of major complications.

A clinical trial by Brumfitt and Pursell (1972)<sup>9</sup> found co-trimoxazole and trimethoprim to be comparable in antibacterial effect, but side-effects occurred in 21% of the co-trimoxazole group against only 8% in the trimethoprim group. When compared with ampicillin and cephalexin, trimethoprim was more effective and had less than half the incidence of side-effects.

In conclusion, therefore, there is little evidence that the three original claims made for co-trimoxazole can be substantiated. The presence of the sulphonamide probably contributes little to the anti-bacterial action of trimethoprim, certainly in the urinary tract, and can cause side-effects. It would be premature to comment on respiratory tract infections, but it would appear that neither the concentrations of the drugs or their concentration ratios are in the range where synergy would be likely to occur.

The future of trimethoprim is, therefore, bright in the short term, but resistance is an increasing problem, and plasmid mediated resistance in particular may prove troublesome in the longer term.

Trimethoprim is a cheap and effective antibacterial drug and should be used alone.

#### Acknowledgements:

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The text of the original dissertation, and comprehensive reference list, is available at the Society's Rooms.