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Sulphamethoxazole/trimethoprim: the first two years

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The combination of sulphamethoxazole and trimethoprim (Bactrim, Septrin) has been available as a chemotherapeutic agent in general use for about two years. It would therefore seem useful at this stage to review published and personal experience with the combination in an attempt to define its probable future role in antibacterial chemotherapy.

Mode of Action

Sulphonamides are thought to produce their antibacterial effect by competing with the natural precursor p-aminobenzoic acid in the formation of folic acid (Fig. 1). In man, the folate pathway requires exogenous folate as its initial substrate so the step blocked by sulphonamides is absent, thus forming the basis for their selective toxicity for bacteria. The next step in the folate pathway is the conversion of folic acid to folinic acid by the enzyme dihydrofolate reductase. Trimethoprim binds strongly to this enzyme in bacteria and blocks this reduction, but binds only weakly to the mammalian enzyme. The presence of blocks at two sequential stages in the folate pathway leads, among other things, to a cessation of DNA synthesis and the death of the bacterium. The sequential nature of the blockage has also been taken to explain the synergistic antibacterial action between sulphonamides and trimethoprim.

The drugs are effective *in vivo*, where folates and folinates abound, because almost all pathogenic bacterial species are unable to absorb and utilize these compounds from an exogenous source (Bushby and Hitchings, 1968).

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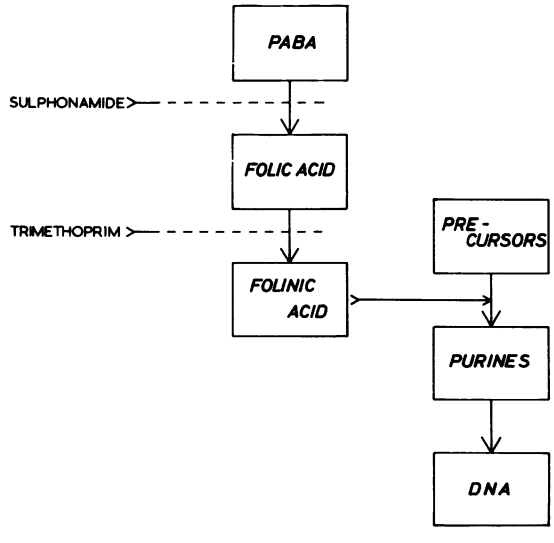

Fig. 1 *Summary of mode of action of sulphonamide and trimethoprim.*

Activity in Vitro

The activity of the individual drugs will be considered first, and then their activity in combination.

TRIMETHOPRIM

The activity of trimethoprim against a number of common pathogens is summarized in Table I from the data of Bushby and Hitchings (1968); Darrell, Garrod, and Waterworth (1968); and Phillips, Ridley, Rimmer, Lynn, and Warren (1970). Trimethoprim has a wide spectrum of antibacterial action and there are examples of both Gram-negative and Gram-positive species in all three of the arbitrary ranges of sensitivity shown. The information given is for typical strains but both small and large variations occur, usually in the direction of resistance. On the whole,

Minimal Inhibitory Concentration (μg/ml)		
<0·25	*0·5-2·0*	*>2·0*
Staph. aureus	*Str. pyogenes*	*Cl. welchii*
Str. faecalis	*Str. pneumoniae*	*N. gonorrhoeae*
		N. meningitidis
H. influenzae	Proteus spp.	*Bacteroides* spp.
Esch. coli	Klebsiella spp.	*Ps. aeruginosa*
Salmonella spp.		*B. pertussis*
Shigella spp.		

Table I *Antibacterial activity of trimethoprim* in vitro

however, resistant strains from those species that are usually sensitive are not common, probably because trimethoprim had not been in widespread use when the data were originally collected.

SULPHONAMIDES

To construct a similar table for sensitivity to sulphonamides would be of little value since acquired resistance to sulphonamides is common among most bacterial species, and in particular in those strains coming from hospital sources. Figure 2 shows data taken from a paper by Brumfitt and Percival (1967) and illustrates this point well. Although the minimal inhibitory concentrations (MIC) shown were estimated using sulphadimidine, it is generally agreed that the sensitivities of bacteria to different sulphonamides are usually in fair agreement. The results in Fig. 2 could therefore be taken as being applicable to a variety of sulphonamides, including sulphamethoxazole.

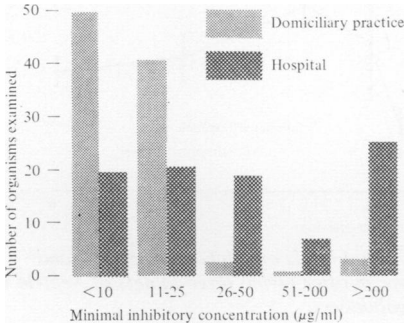

Fig. 2 *Minimum inhibitory concentrations of sulphadimidine for organisms from acute urinary infections in general practice patients and urinary infections in hospital patients.*

The sensitivity of various bacterial pathogens to sulphonamides varies widely but sensitive strains have MICs of < 50 μg/ml. Infections with organisms having an MIC above this figure are less likely to be eradicated by sulphonamide therapy (Williams and Leigh, 1966), and treatment is particularly likely to fail if the MIC is > 200 μg/ml.

COMBINED ACTIVITY

The difficulty in reviewing the action of the sulphamethoxazole/trimethoprim combination is that not only has the activity of the individual components to be described but that additive or synergistic effects, ie, potentiation, between the two drugs must also be considered.

Synergism

That synergism between these two drugs occurs has been shown by Bushby and Hitchings (1968) and Darrell *et al* (1968), some of the information being summarized in Table II. Although the degree of potentiation may vary for different strains of a particular species, the table shows the results for typical strains and gives the order of potentiation to be expected. An important point is that the degree of potentiation which occurs depends on the ratio of the concentrations of sulphamethoxazole and trimethoprim used, and tends to be maximal when the drug ratio of the concentrations is similar to that of their respective MICs (Bushby and Hitchings, 1968). Thus, for a range of organisms sensitive to both drugs the ratio would be about 20 parts of sulphamethoxazole (MICs 5-50 μg/ml) to one part of trimethoprim (MICs 0·25-2 μg/ml), although it may vary widely with individual species. *Neisseria gonorrhoeae* is an exception in which the ratios of the MICs of sulphamethoxazole and trimethoprim are completely reversed, the organism sometimes being more sensitive to sulphonamides than to trimethoprim.

x2-x8 Potentiation	*x16-x64 Potentiation*
Staph. aureus	*N. gonorrhoeae*
Str. pyogenes	*Pr. mirabilis*
Str. pneumoniae	
H. influenzae	
Esch. coli	
Klebsiella spp.	
Salmonella spp.	
Shigella spp.	

Table II *Potentiation of activity of trimethoprim by sulphonamide* in vitro[1]

[1]Sulphonamide : trimethoprim ratio 9:1 (except for *N. gonorrhoeae*) Data from Darrell *et al* (1968).

Evidence of synergistic activity has also been found *in vivo* in experimental infections in mice (Bushby and Hitchings, 1968; Bohni, 1969) and in the treatment of gonorrhoeae (Table III). Typical strains of *N. gonorrhoeae* are resistant to trimethoprim (the MICs being almost always > 2 μg/ml) and treatment with this drug alone results in uniform failure. Treatment with a sulphonamide alone

Drug and Daily Dose (mg)		*Cases Treated and Followed*	*Failures*
Trimethoprim	*Sulphatriad*		
400	—	10	10
—	4,000	13	9
400	4,000	37	4
200	4,000	9	0
100	4,000	5	0

Table III *Potentiation of activity* in vivo *of trimethoprim by sulphonamide in the treatment of gonorrhoea*[1]

[1]Data from Csonka and Knight (1967)

produces a cure rate of only about 30% because of the prevalence of acquired sulphonamide resistance. Combined therapy gives a high cure rate similar to that produced by penicillin G. This effect seems to be greater than that produced by simple addition of activities and must surely represent true synergy.

Addition

Apart from the occurrence of synergy another reason for the administration of a sulphonamide with trimethoprim is that the additive action of the two drugs makes the administration of a lower dose of both drugs possible, thus reducing the incidence of side effects.

Two further reasons for combined administration are the prevention of acquisition of resistance to trimethoprim, and the conversion of the bacteriostatic activity of both drugs to a bactericidal action.

Prevention of resistance

Bacteria can be 'trained' to trimethoprim resistance by serial subculture on trimethoprim-containing medium (Darrell *et al*, 1968). The development of this resistance is reduced by the addition of sulphonamide to the medium, the effect being less noticeable when the organism used is resistant to sulphonamides.

Bactericidal activity

The sulphonamides are well known to be bacteriostatic, and trimethoprim is similar in this respect. When both drugs are used in combination, however, they may be bactericidal (Bushby, 1969a). How often this effect occurs with individual species or strains is not known, but it probably occurs often enough to make it yet another indication for combining trimethoprim with sulphonamide rather than using it alone.

Antagonism between sulphonamides and trimethoprim has not been demonstrated (Bushby, 1969b), nor between trimethoprim and other antibiotics.

Pharmacology

ADMINISTRATION

The drugs are presented in tablets (Septrin[1]) or drapsules (Bactrim[1]) containing 400 mg of sulphamethoxazole and 80 mg of trimethoprim, ie, a sulphonamide/trimethoprim ratio of 5:1. This ratio of administration is used because it was found by experience that it produced a ratio in the blood of 20:1 (see Fig. 3, for example) and, as mentioned above, this is most likely to give maximal potentiation for a wide variety of organism.

[1]UK only

ABSORPTION

The uptake of both sulphamethoxazole and trimethoprim from the intestinal tract following oral administration is rapid and good blood levels are obtained. The results of giving Septrin to four fasting volunteers (Fig. 3) illustrates this and also shows that there is remarkably little deviation from the mean values produced. More important, uptake of both drugs in patients under treatment is also satisfactory (Fig. 4). Some accumulates after the initial dose, the mean levels being higher with repeated administration. Both drugs are excreted by the kidneys, trimethoprim largely in an unchanged form, producing high urinary levels (see Brumfitt, Faiers, Pursell, Reeves, and Turnbull, 1969). As would be expected, blood levels of both drugs are higher in patients with renal failure.

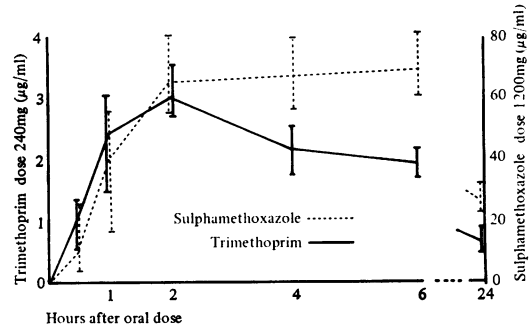

Fig. 3 *Blood levels of sulphamethoxazole and trimethoprim after giving three tablets of Septrin to four fasting volunteers.*

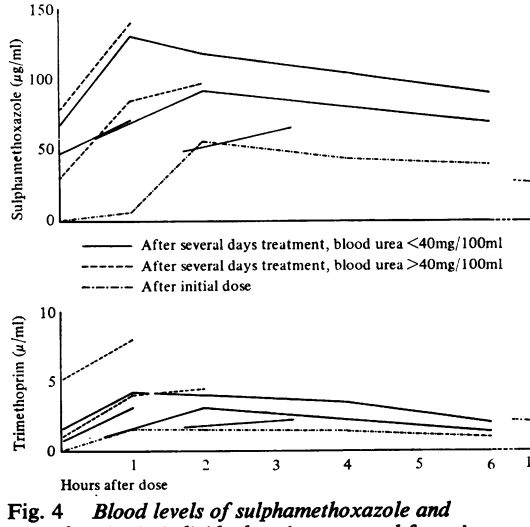

Fig. 4 *Blood levels of sulphamethoxazole and trimethoprim in individual patients treated for urinary infection with Septrin. (Dose sulphamethoxazole 800 mg, trimethoprim 160 mg, twice daily.)*

DISTRIBUTION

The standard preparation provides a fixed ratio of the two drugs. There are, however, a number of reasons why having a fixed combination is far from ideal. Of the sulphonamides available, sulphamethoxazole was chosen for combination with trimethoprim because of its high activity and similar blood half-life. Although the ratio of drugs in the blood may be suitable for treating many infections, the organisms are more usually in other tissues or body fluids. A study of the pharmacology of sulphamethoxazole and trimethoprim indicates that, both directly and inferentially, the ratio of the two drugs found in the blood will almost certainly not be maintained in the tissues. Data for concentrations in body fluids, some unpublished, collected from a variety of sources bear out this point (Table IV). Trimethoprim base is lipid soluble and seems to diffuse throughout the tissues of the body. Data for animal tissues (Table V) show that, in general, trimethoprim is concentrated in tissue, whereas tissue levels of sulphamethoxazole are lower than those found in the serum. Thus, in the treatment of individual tissue infections, it may be necessary to supplement the sulphonamide dosage if a satisfactory ratio between the two drugs is to be maintained.

Although typical ratios of urinary concentrations of the drugs are not ideal, the overall antibacterial activity is usually more than adequate to deal with urinary pathogens. The ratio between the drugs may be still further disturbed, however, when renal function is impaired, since the renal handling of sulphamethoxazole and trimethoprim differs (Sharpstone, 1969). This may also affect the relative serum levels. Apart from the differences that exist in the tissues the optimum serum ratio of 20 : 1 is obviously not ideal for all organisms although minor deviations from it are probably of little consequence. In the case of some bacteria the optimum ratio may be very different or even reversed, eg, *N. gonorrhoeae*, and in such cases it would seem logical to supplement therapy with whichever of the two drugs is appropriate.

Even where the ratio of concentrations between a sulphonamide and trimethoprim are satisfactory, there are instances where, for pharmacological reasons, sulphamethoxazole may not be the ideal sulphonamide. Trimethoprim is concentrated in the prostatic fluid of dogs, whereas sulphamethoxazole is found in a concentration far less than that found in the serum (Reeves and Ghilchik, 1970). Of the other sulphonamides available, sulphadimidine is excreted best in prostatic fluid (Winningham and Stamey, 1970), and a combination of this drug with trimethoprim may therefore be better for treating prostatic infection, although MICs to sulphadimidine are in general higher than to sulphamethoxazole.

In spite of these criticisms of the fixed ratio, the marketing of a number of preparations with different ratios would produce complexities beyond the comprehension of many prescribers and could lead to serious therapeutic errors. All that can be said at present is that for serious and difficult infections in

	Trimethoprim Concentration	*Animal*	*Sulphamethoxazole Concentration*	*Animal*
Urine	×100 (approx.)	Man	×5 (approx.)	Man
Cerebrospinal fluid (uninflamed meningitis)	×0·5	Man	×0·3	Man
Sputum	×2·5	Man	×0·1	Man
Milk	×1	Man	?	
Bile	×2	Man	?	
	×15	Dog	?	
Prostatic fluid	×3	Dog	×0·25	Dog

Table IV *Trimethoprim/sulphamethoxazole concentrations in body fluids relative to serum level*

	Trimethoprim Tissue/Serum Ratio	*Animal*	*Sulphamethoxazole Tissue/Serum Ratio*	*Animal*
Liver	6·2	Rat	0·3	Rat
	3·2	Mouse	0·25	Rabbit
Lungs	2·0	Rat	0·4	Rat
	17·5	Mouse	0·5	Rabbit
Heart	1·2	Rat	0·35	Rat
	6·5	Mouse	0·6	Rabbit
Kidneys	7·3	Rat	0·6	Rat
	7·6	Mouse	2·4	Rabbit
Brain	0·08	Rat	0·05	Rat
			0·25	Rabbit

Table V *Trimethoprim/sulphamethoxazole concentrations in animal tissue relative to serum levels*[1]

[1]Data from Bushby and Hitchings (1968) and other sources.

particular tissues the fixed ratio may not provide ideal therapy and supplementation of treatment by one or other drug may be advisable.

PARENTERAL ADMINISTRATION

Because of its high degree of activity against a wide range of pathogens, the combination would seem suitable for treating serious infections if it could be administered parenterally. Unfortunately, no preparation is generally available although trimethoprim lactate alone can be obtained for treating special cases, and parenteral sulphonamide preparations are generally available. Should intravenous therapy be used trimethoprim should not be mixed with sulphonamide in the infusion fluid since they may be pharmaceutically incompatible.

Therapeutic Uses

Numerous reports on the use of sulphamethoxazole/trimethoprim in the treatment of infective diseases and some non-infective diseases have appeared. Some references are listed in Table VI. There is no space here for detailed analysis of published findings but, in summary, the reports are generally favourable. There are, however, some specific points of interest arising from the treatment of certain infections, notably urinary infections, respiratory infections, and gonorrhoea.

Urinary infections arising in both domiciliary and hospital patients have been successfuly treated. We have found a particularly high cure rate in the treatment of bacteriuria in pregnancy (Williams, Brumfitt, Condie, and Reeves, 1969), even in those patients in whom treatment with another agent had already failed. In view of the fact that pregnancy pyelonephritis is as common in women in whom treatment for bacteriuria has failed as in untreated women (Williams, Brumfitt, Condie, and Reeves, 1969), confirmation of our findings would seem to represent an advance in therapy. Unfortunately, because of teratogenicity in animals given high doses of trimethoprim (Udall, 1969), its use in pregnancy is not recommended and such confirmation is unlikely to be forthcoming in the near future. In the relatively small number of women we treated, all after the 12th week of pregnancy, no congenital malformations attributable to the drug were found in a careful follow-up study.

As mentioned previously, sulphonamide resistance is common among urinary pathogens isolated from hospital patients (Fig. 2) and, hardly surprisingly, the cure rate with sulphonamide therapy allocated on a random basis as treatment for urinary infection is low (Table VII, Reeves, Faiers, Pursell, and Brumfitt, 1969). Sulphamethoxazole/trimethoprim gave a higher cure rate than ampicillin, although the difference was not statistically significant. Most of the infecting organisms were sensitive to trimethoprim and this may have been the reason for the good results.

Because patients with urinary infections can be divided into more or less homogeneous groups, a more precise analysis of the results of treatment can be meaningful. Only in such patients has any attempt been made to define the degree of potentiation *in vitro* in determining the outcome of therapy. The results of Grüneberg and Kolbe (1969)

Treatments Described	*Disorder*	*Reference*
Many	Urinary infection	Allison *et al* (1969) Cox and Montgomery Grüneberg and Kolbe (1969) Reeves *et al* (1969) O'Grady *et al* (1969) Williams *et al* (1969)
	Respiratory infection	Drew *et al* (1967a and b) Hughes (1969) Hughes *et al* (1969) Lal and Bhalla (1969) Pines *et al* (1969)
	Venereal disease	Csonka and Knight (1967) Csonka (1969) Caroll and Nicol (1970) Wright and Grimble (1970)
Relatively few or single treatments	Coliform meningitis	Morzaria *et al* (1969)
	Coliform endocarditis	Fowle and Zorab (1970)
	Coliform tissue infection	
	Typhoid cases and carriers	Akinkugbe *et al* (1968) Brodie *et al* (1970) Kamat (1970) Farid *et al* (1970)
	Cholera carriers	Gharagozloo *et al* (1970)
	Acute brucellosis	Lal *et al* (1970)
	Staphylococcal osteomyelitis	Pugsley *et al* (1969) Craven *et al* (1970)
	Gram-negative bacteraemia	Noall *et al* (1962) Cooper and Wald (1964) Darrell *et al* (1968)
	Nocardiosis	Marcovitch & Harvey (1970) Murray and Mahgout (1970)
	Ulcerative colitis	Savidge (1969)
	Prophylaxis in leucopenia	Grüneberg *et al* (1970)

Table VI *Some reported therapeutic uses of sulphonamide/trimethoprim*

Treatment	*No. of Patients Treated*	*One Week after End of Treatment*	*No. of Patients Treated and Cured*	*Four to Five weeks After End of Treatment*
Sulphamethoxazole-trimethoprim 5:1	41	35 (85%)[1]	27	18 (67%)
Ampicillin	30	21 (70%)[1]	27	14 (52%)
Sulphadimidine	35	14 (40%)	26	4 (15%)

Table VII *Results of treating hospital-acquired urinary infection*

[1]P = 0·102 for the difference between the results at one week for sulphamethoxazole-trimethoprim and ampicillin.

suggested that the chances of successful treatment of a urinary infection with the combination increased with increasing *in vitro* potentiation of sulphamethoxazole by trimethoprim, although a statistical significance was not reached.

Successful results have also been claimed in respiratory infections, including the prevention and treatment of the exacerbations of chronic bronchitis and the treatment of pneumonia. A disturbing aspect of respiratory infections has been the description of the isolation of *Haemophilus influenzae* highly resistant to trimethoprim from patients on long-term treatment with the combination (Waterworth, 1969). In view of the possible importance of sulphonamide in preventing the emergence of resistance to trimethoprim, the relatively low sputum levels of sulphamethoxazole (Table IV) may be relevant. Furthermore, May (1969) has described the early relapse of infection in patients treated for chronic bronchitis and suggested that the therapy appears to be bacteriostatic in nature. The levels of sulphamethoxazole in sputum may again be relevant. In fairness it must be mentioned, however, that other workers have clearly demonstrated eradication of pathogens from the sputum (Hughes, 1969) and a comparatively low early recurrence rate (Pines, 1970).

The use of sulphamethoxazole/trimethoprim for the treatment of gonorrhoea has definite attractions. In addition to producing a high cure rate, the combination has no activity against the spirochaetes causing syphilis and there is therefore no chance of masking a concomitant syphilitic infection. A disadvantage is that at least four days' treatment must be taken if conventional doses are employed.

Two other particular reports are of interest. One is the use of the combination in ulcerative colitis, where the effect produced might be associated with the recently described immunosuppressive activity of trimethoprim (Ghilchik, Morris, and Reeves, 1970). The other is a report of successful use in the prevention of infection in leucopenic patients (Grüneberg, Emmerson, Prankerd, and Souhamie, 1970). Although some bacteria commonly causing opportunistic infections are 'resistant' to sulphamethoxazole/trimethoprim, it should be remembered that the combination is only required to prevent establishment of infection from a small, initiating inoculum whereas sensitivity or resistance is usually judged on the basis of what is needed for treating the large number of organisms in established infection.

Toxicity

The toxicity of sulphamethoxazole/trimethoprim can be that of the individual components of the combination and perhaps also the result of both drugs acting to produce a single effect. Although much of the recent literature concentrates on the mechanism of the toxic potential of trimethoprim (e.g. Kahn, Fein, and Brodsky, 1968), it must be remembered that large amounts of sulphamethoxazole are being administered in the combination, and sulphonamide side effects will also occur. Minor side effects (skin rash, nausea, vomiting, glossitis) occur occasionally after giving the combination (Reeves *et al*, 1969; Williams *et al*, 1969). On the evidence available, serious haematological side effects have only occurred very rarely during the administration of what is conservatively estimated to be 40,000,000 tablets of the combination so far. Thrombocytopenia seems to be the most common serious blood defect (Handley, 1969), but as both this and neutropenia are found in ill patients, particularly those with virus infections, the significance of their presence after drug administration is hard to assess. There seems to be a particular association between thrombocytopenia and heart failure (Palva, Salokannel, and Takkunen, 1970). The administration of the combination to patients with heart failure might be better avoided, especially if a thiazide drug is also being given. It has also been suggested that neutropenia is particularly likely to occur when the combination is given to patients within 60 days of receiving a homotransplanted kidney (Hulme and Reeves, unpublished data).

Laboratory Control

DISC SENSITIVITY TESTING

The practical aspects of this topic have already been discussed by Waterworth (1969). The importance of the correct choice of medium and control of inoculum size should be emphasized. It would appear that Oxoid DST agar containing lysed horse blood

is a generally suitable medium. Very heavy inocula should be avoided since they selectively reduce inhibition zones to sulphonamides.

A more controversial problem is the choice of suitable discs since bacteria can be tested either against each drug separately or both together in a combined disc. The former method gives the most information about the activity of the individual drugs against the bacteria but significant and useful degrees of synergy may well be missed. Although synergy cannot be conclusively demonstrated by the use of a combined disc alone, its effect (and hence its therapeutic potential), if present at the ratio of the two drugs employed, is more likely to be detected. There is no doubt that ideally both methods should be employed and this can be recommended for serious or difficult infections. It is, however, time consuming and extravagant in materials and, for simple infections (for example a lower urinary tract infection) a combined disc test determining overall sensitivity to the combination is probably sufficient.

Further argument on this point is fruitless at present since more work is needed, for example, in assessing the importance of initial resistance to sulphonamide in determining the development of resistance to trimethoprim during therapy, or the occurrence of potentiation when the organism is partially or fully resistant to one or both drugs. Until points like these are resolved by carefully conducted clinico-microbiological studies the formulation of sensitivity testing procedures cannot usefully be finalized.

DETERMINATION OF MINIMAL INHIBITORY CONCENTRATION

For both sulphonamide and trimethoprim an agar dilution technique with multiple loop inoculation (Darrell *et al*, 1968) can be used. Careful control of the inoculum size is essential for reproducible results and we use an inoculum concentration of only 10^3-10^4 organisms/ml to ensure meaningful sulphonamide minimum inhibitory concentrations.

SERUM LEVELS OF DRUGS

The estimation of sulphonamide in body fluids can be rapidly and simply done by a chemical method (Bratton and Marshall, 1939, as described and modified by Varley, 1967). Trimethoprim can be estimated by a rather complex microbiological method fully described by Reeves and Ghilchik (1970). Although a simpler method, more suitable for clinical use, has been described (Pechère, 1970) it still requires a full evaluation by comparison with standard methods. A spectrofluorimetric method has also been described (Schwartz, Koechlin, and Weinfeld, 1969) a micromodification of which apparently gives results in good agreement with the microbiological assay (A. S. E. Fowle, personal communication). But it is a time-consuming procedure, needing specialized apparatus and requiring careful attention to detail for reproducible results.

Prospect

The results of laboratory and clinical studies to date have shown sulphamethoxazole/trimethoprim in combination to be a chemotherapeutic agent of wide application. Its place in the chemotherapeutic armamentarium of the future will depend on three main factors considered in relationship to other drugs available: (1) the ease of development of resistant strains in domiciliary and hospital acquired infections; (2) the frequency and seriousness of side effects; and (3) teratogenicity.

These problems will only be resolved by the continuing use of the combination under careful observation and control. The evidence available so far does not suggest that it is any more toxic than many other commonly used antibacterial agents.

From the point of view of practical chemotherapeutics other problems remain to be solved, in particular the importance of initial sulphonamide resistance in infecting bacteria treated with the combination, and the part played by synergic activity in controlling and eradicating infections. Until these problems are resolved by clinicomicrobiological studies the correct laboratory control of therapy with the combination will remain partly conjectural.

Finally, it would seem that the administration of a fixed ratio of the two drugs is not ideal for treating all infections. For treating infections in certain sites, or infections caused by certain organisms, separate tablets of trimethoprim or sulphamethoxazole would be of value so that the ratio with sulphonamide could be altered or a different sulphonamide be employed.

I wish to thank Professor R. E. O. Williams for his helpful criticism in compiling this review.

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