DO SULPHONAMIDE-TRIMETHOPRIM COMBINATIONS SELECT LESS RESISTANCE TO TRIMETHOPRIM THAN THE USE OF TRIMETHOPRIM ALONE?

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Introduction

Trimethoprim (TMP) is a diaminopyrimidine with a high specificity for bacterial dihydrofolate reductase (DHFR) (Burchall and Hitchings, 1965). Since the initial synthesis of TMP about 20 years ago, no other diaminopyrimidine has been developed with such potency and specificity for the bacterial reductase.

Trimethoprim has been used widely since 1969 in the UK, combined with sulphamethoxazole (SMX) as co-trimoxazole (Co-T) or, recently, with sulphadiazine or sulphamoxole. Trimethoprim has been available alone in Finland since 1975 and, since 1979, in other countries including Great Britain, Denmark, Holland, West Germany, Norway, Switzerland, Ireland, Iceland, Kenya, Nigeria and the USA.

When co-trimoxazole was introduced in 1969, the two components were thought to present a unique combination for two principal reasons: remarkable synergy between them and the ability to suppress the development of resistance (Bushby and Hitchings, 1968). Thus, although TMP was known to possess potent antibacterial activity in its own right (Hitchings, 1969), it was considered a sulphonamide potentiator (Bushby and Hitchings, 1968; Hitchings, 1970) and there was little evaluation of its use clinically as a single substance. In considering whether the use of TMP alone can be as clinically effective and as safe as the combination in relation to the emergence of resistance, two issues arise: (1) how relevant is synergy between the components in vivo to clinical use, and (2) does the suppression of resistance that is demonstrable in vitro occur in vivo? The first question has largely been answered: first, in models of urinary-tract infection
with concentrations of each agent that occur during therapy, the activity of TMP is so
dominant over the sulphonamide that the potential for synergy between them does not
occur (Anderson et al., 1974; Greenwood and O'Grady, 1976; Greenwood, 1979;
Lacey, Rogerson and Stokes, 1980b); second, the concentration of TMP in the body
during therapy is sufficient to inhibit most of the pathogens in question (Hansen et al.,
1973; Wilkinson and Reeves, 1979); and third, TMP alone has been found to be as
effective as the combination in treating acute infections of the urinary tract and
respiratory tract (Brumfitt and Pursell, 1972; Koch et al., 1973; Kasanen et al., 1978;
Lacey et al., 1980a; Mabek and Vejlsgaard, 1980; Trimethoprim Study Group, 1981).
Trimethoprim is also effective in treating patients with typhoid fever (McKendrick,
Geddes and Farrell, 1981). In each of these studies, the doses of TMP were similar to
the amount of drug in the combination. Thus, whilst synergy between TMP and SMX
readily occurs in vitro, this does not seem to be a requirement for success in treating
many acute infections. That TMP alone is clinically effective is not surprising because
many of the pathogens that have been eliminated by the combination are highly
resistant to SMX (Greenwood, 1980), and the combination does not usually produce
synergistic inhibition of these cultures in vitro (Hamilton-Miller, 1979; Lacey, 1979a).
It is beyond the scope of this review to discuss the use of the combination in treating
infections caused by bacteria relatively resistant to TMP, e.g., Neisseria gonorrhoeae,
when therapeutic success might depend on synergy between SMX and TMP.

The important outstanding question is whether the prescription of a sulphonamide
simultaneously with TMP does prevent bacteria from acquiring resistance to TMP.

**Mechanism of action of trimethoprim and sulphonamides**

Sulphonamides interfere competitively with the transformation of para-aminobenzoic acid (PABA) by dihydropteroate synthetase in the first part of the pathway leading
to the formation of tetrahydrofolic acid (Burchall, 1979). Small amounts of PABA
antagonise the inhibitory effect of sulphonamides (Lacey, 1979a). There is little
PABA in normal human tissues, but frank pus may contain enough to reduce the
activity of SMX. Trimethoprim binds to DHFR (Burchall, 1979); the affinity of TMP
is much higher for the bacterial enzyme than for the human analogue (Burchall, 1969).
The effect of SMX and TMP ultimately reduces the pool of tetrahydrofolic acid
available as a co-factor for amino-acid metabolism and DNA synthesis (Burchall,
1969, 1979). These mechanisms are summarised in the figure.

Whilst the two drugs are said to act by sequential blockade (Darrell, Garrod and
Waterworth, 1968), this is not necessarily the explanation for synergy (Webb, 1963;
Poe, 1977). When a particular pathway is completely inhibited at a point along it,
inhibitors acting at another point may not be able to increase the degree of inhibition.
Thus, when bacteria are exposed to concentrations of TMP adequate for inhibition of
bacterial growth, addition of SMX or a further increase in the concentration of TMP
does not increase the rate of bacterial killing (Lacey et al., 1980b).

Poe (1977) suggested that synergy results from the simultaneous binding of TMP
and SMX to DHFR, when the drugs are present in sub-inhibitory concentrations.
Byford (1979) has confirmed this finding with Poe's purified Escherichia coli enzyme
(table I). More work is needed to establish the general application of these
observations because they imply that sulphonamides have two points of action.
Meanwhile, the proposed actions of TMP and SMX may account for the apparently conflicting evidence for or against synergy of TMP and SMX in tests with sulphonamide-resistant bacteria (Gruneberg, 1975, 1979). In bacteria highly resistant to sulphonamides, due, for example, to exclusion of the molecule from the cell, synergy should not occur. In tests with cultures selected for sulphonamide resistance in vitro, or with clinical strains resistant to low concentrations of sulphonamides where the resistance may be due to alterations in dihydropteroate synthetase, synergy may still occur (Lacey, 1979a). In these, SMX could still potentiate TMP inhibition of the DHFR. The observation that synergy between SMX and TMP may occur in the presence of pus (Edmunds, 1978) although activity of sulphonamides is reduced, is explained by the presence of PABA which will neutralise only the first point of action of sulphonamides. However, Then (1977) has claimed that the presence of PABA abolishes synergy between TMP and SMX. We have studied this in two ways (Lacey, 1979a). First, the effect of PABA on synergy between sulphonamides and TMP was assessed by determining MICs in media supplemented with a range of concentrations of PABA. In a typical culture 0.1–1.0 µg PABA/ml raised the MIC for sulphonamides from 10 µg/ml to 100–1000 µg/ml, but synergy can still be clearly demonstrated. At

Table I

Effect of trimethoprim and a sulphonamide (sulphapyridine) on the inhibition of E. coli dihydrofolate reductase

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Number of estimations</th>
<th>Reaction rate*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme, substrate</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Enzyme, substrate and 2 × 10⁻⁴mM TMP</td>
<td>10</td>
<td>72.5</td>
</tr>
<tr>
<td>Enzyme, substrate and 20mM sulphapyridine</td>
<td>10</td>
<td>95.8</td>
</tr>
<tr>
<td>Enzyme, substrate, 2 × 10⁻⁴mM TMP and 20mM sulphapyridine</td>
<td>14</td>
<td>52.6†</td>
</tr>
<tr>
<td>Enzyme, substrate, 2 × 10⁻⁴mM TMP, 20mM sulphapyridine and 20mM PABA</td>
<td>15</td>
<td>50.6†</td>
</tr>
</tbody>
</table>

* 100 = Maximum enzyme activity; arbitrary units determined by estimation of reaction rate in centrifugal analyser (Byford, 1979).
† Difference not significant: value for t = 0.44; p > 0.1.
very high concentrations of PABA (25 μg/ml) synergy is destroyed. The second approach was to study whether sulphonamides potentiate the inhibitory effect of TMP on the purified E. coli DHFR in the presence of PABA. There was indeed potentiation even in the presence of PABA (Byford, 1979, and see table I). Then's (1978) findings may be attributable to the use of inappropriately high concentrations of PABA or the failure to use optimal ratios of the drugs to detect synergy. Golde, Bersch and Quan (1978) have shown that human and murine haemopoietic cell cultures were each inhibited by SMX. The inhibition was presumably caused by binding of SMX to DHFR because it was reversed by addition of folic acid.

Uncertainty about the points of action of sulphonamides and TMP makes any prediction of the development of resistance during clinical use speculative. Because both sulphonamides and TMP may act on the same step in the metabolic pathway, there may be no protective effect from using the combination rather than TMP alone. For example, "mutant" enzymes might simultaneously be resistant to both drugs, or alternative pathways may bypass this target. Even though both agents can act sequentially, there is no reason to think that the presence of one agent will necessarily protect the other against acquired resistance. Although the appearance of resistant mutants may be prevented in vitro, the crucial question, as discussed later, is whether these experiments are clinically relevant.

General mechanisms of antibiotic resistance

The frequent isolation of resistant bacteria may reflect the spread of one or a few clones of rare resistant organisms, or may be a result of resistance arising de novo at high frequency in many different strains. Resistant organisms may grow slightly less rapidly than their sensitive counterparts; this probably accounts for their disappearance in vivo when an antibiotic is withdrawn (Lowbury, Babb and Roe, 1972; Lacey, Lewis and Grinsted, 1973; Lilly and Lowbury, 1978). A consequence of the presence of additional DNA in multi-resistant bacteria may be their reduced growth rates in antibiotic-free media (Lacey and Chopra, 1975; Helling, Kinney and Adams, 1981). Because resistant cells may be atypical in general properties, their virulence may be altered. In assessment of the different selection pressures of TMP alone and TMP–SMX combinations, knowledge of antibiotic prescribing generally and the virulence of the resistant cultures is relevant. Measures aimed at preventing cross infection are also important. Many surveys of resistance do not consider these aspects.

After exposure of a sensitive culture to an antibiotic at a concentration of about 4–10 times that needed to inhibit the growth of the majority of the bacteria, a few cells will multiply after incubation. These mutants contain aberrant DNA that formed spontaneously before antibiotic exposure and typically show: (1) growth rate slower than that of the wild strain; (2) instability of the change; (3) heterogeneous colonial properties; (4) cross resistance to chemically related antibiotics; and (5) a possible requirement for co-factors for growth (see, e.g., Lacey, 1969). Most antibiotics select this type of atypical derivative in vitro at frequencies characteristically of 10⁻⁶–10⁻⁹. However, mutants resistant to fusidic acid (Evans and Waterworth, 1966; Lacey, 1979b), and rifampicin can have properties comparable to those of wild strains.
Is the use of TMP particularly prone to select resistance by mutation?

Darrell et al. (1968) studied serial transfers of four cultures of the Enterobacteriaceae on ditch plates containing TMP. After 25 subcultures of a light inoculum of each, increase of resistance was not detected but, with a heavy inoculum, each culture yielded resistant variants, most with normal colonial appearance. The virulence of resistant organisms selected by serial transfer in the presence of TMP is not known (Darrell et al., 1968; Breeze, Sims and Stacey, 1975; Grunberg and Beskid, 1977). These findings suggest that there are a few cells in a culture resistant to low concentrations of TMP; these in turn contain a minority of cells resistant to higher concentrations, so that with serial transfer highly resistant derivatives are obtained. The biochemical mechanism of this resistance is not known, although it is known to be determined by a cluster of altered genes (Breeze et al., 1975). When a sulphonamide is incorporated into the medium in addition to TMP, the development of this resistance is inhibited (Darrell et al., 1968; Grunberg and Beskid, 1977). However, these manipulations may not represent events within patients treated with TMP and the nature of TMP resistance found in clinical isolates may differ from that in cultures exposed to TMP in vitro.

During treatment of infection with TMP the concentration of the drug at the site of the infection soon exceeds that needed to inhibit the growth of most pathogens. Bacteria in vivo are not exposed to gradually increasing concentrations of TMP over weeks. Moreover, if TMP is added in therapeutic concentrations to E. coli suspended in serum, urine or nutrient broth, resistant variants of E. coli do not emerge (Lacey et al., 1980b). However, Streptococcus faecalis can develop resistance to TMP in one step in vitro (Lewis and Lacey, 1973). Because this organism is always highly resistant to sulphonamides, there is little opportunity for the administration of sulphonamides to protect the cells from acquiring resistance to TMP.

Thymine or thymidine-requiring bacteria

One relatively rare type of mutational resistance to TMP that has been shown to be important clinically concerns thymine or thymidine-requiring mutants (Stacey and Simson, 1965; Amyes and Smith, 1975). These bacteria possess a presumptive abnormality in the chromosomal gene coding for thymidylate synthetase and must utilise thymine directly, with the point of action of TMP and sulphonamides by-passed. These mutants require thymine or thymidine for growth and are highly resistant to SMX and TMP. Lysed horse blood enhances the antibacterial activity of SMX and TMP because horse red cells contain an enzyme that phosphorylates thymidine and, to a lesser extent, thymine (Ferone et al., 1975).

Thymidine or thymine-requiring mutants have caused urinary, respiratory and wound infections and also salmonellosis. These patients had all been treated with co-trimoxazole (Barker, Healing and Hutchinson, 1972; Lacey and Lewis, 1973; Okubadejo and Maskell, 1973; Tapsall, Wilson and Harper, 1974; Maskell, Okubadejo and Payne, 1976; George and Healing, 1977; McCarthy et al., 1977; Sparham, Lobban, and Speller, 1978). The thymine and thymidine found in the associated lesions has presumably been formed from bacterial and from leukocyte DNA degradation.

However, infections caused by these mutants are rare because the mutation rate to
thymine (thymidine) requirement is low; when thymidine is removed from the environment, the mutants die (Stokes and Lacey, 1978). Because thymidine is found in significant amounts only in infected human material (Notterbrock and Then, 1977) these mutants have not caused cross infection. Furthermore, they may be less pathogenic than wild strains (Smith and Tucker, 1976). However, the significance of these mutants is fourfold: (1) their isolation from purulent clinical material indicates that concentrations of thymidine or thymine sufficient to support growth of mutants are present in vivo. These substances might impede the activity of SMX and TMP in frankly purulent tissues; (2) they are a rare example of resistance acquired by manipulation in vitro being equivalent to that appearing in vivo; (3) the presence of SMX has not prevented the appearance of such mutants during therapy with TMP; (4) these mutants, like others, are defective and survive poorly, unless specific co-factors are present.

Plasmid-mediated antibiotic resistance and transposons

Most clinically important acquired resistance is mediated by bacterial plasmids (Timmis and Pühler, 1979). Plasmids may carry genes with functions other than coding for antibiotic resistance, e.g., virulence, specific degrading activity and prophage function. The two essential attributes of plasmids are their physical separation from bacterial chromosome and their non-essential nature generally that permits changes in their DNA without endangering the viability of the cell, as could alterations of many chromosomal loci.

The origins of plasmids are not known with certainty. They may contain DNA sequences that resemble those of the bacterial chromosome or bacteriophages, and they may have evolved from these replicons. Alternatively they may have evolved in situ; however, until such a structure has become functional it is difficult to anticipate any selective advantage conferred on the cell by its existence and it would tend to be lost. An important practical point is that it has not been possible to construct in vitro a totally new plasmid out of small molecules. Modification of existing plasmids is easily achieved by the use of restriction endonucleases that can cleave and re-assemble DNA at specific loci (Timmis and Pühler, 1979). Plasmids therefore do not arise de novo within the cell as a result of antibacterial therapy, but pre-date its use. Before bacteria are exposed to antibiotics, plasmids are found usually in the minority, perhaps 1 in $10^3$–1 in $10^{12}$ cells of a species; only a proportion of these carry genes determining antibiotic resistance.

The mechanism of most naturally occurring plasmid-borne antibiotic resistance is drug inactivation by enzymes, e.g., $\beta$ lactamases, aminoglycoside adenylations, acetylation and phosphorylation, or chloramphenicol acetylation. (Tetracycline resistance is, however, attributable to exclusion of the antibiotic from the cell (Chopra, 1976), and erythromycin resistance to abnormal ribosomes (Weisblum, 1971).) The inactivating enzymes are highly specific for the antibiotic in question; the sequence of DNA bases determining these proteins must also be specific and cannot arise rapidly de novo. Thus it is not possible to select bacteria with such naturally occurring resistance mechanisms by plating dense sensitive inocula on media containing antibiotics. Even if a rare mutant is present in such an inoculum, it may not be able to express its enzyme sufficiently quickly and in sufficient amounts to ensure its survival and replication. A
relatively few genes determining resistance have seemingly evolved over a very long time, and the use of antibiotics selects these rare cultures in vivo at the expense of sensitive organisms. There is epidemiological evidence for transfer of plasmids between members of one bacterial species and perhaps sometimes to other species. In vitro there are numerous ways that genes can be so transferred (Broda, 1979). Identical genes coding for specific resistance mechanisms may be on different plasmids, and even on the chromosome within different isolates of a bacterial species. An extremely efficient mechanism can achieve this; it is called transposition. The gene in question is surrounded by a specific sequence of DNA bases (insertion sequences). These are common to many loci on the same and on different replicons so that a small segment of DNA coding for antibiotic resistance can “jump” between different replicons without their destruction. This mechanism was first described in 1974 for ampicillin resistance in E. coli (Hedges and Jacob, 1974) and is now thought to occur generally. Changes in plasmid carriage combined with transposition accounts for most of acquired antibiotic resistance (Heffron et al., 1975; Heffron, Rubens, and Falkow, 1975; Barth and Datta, 1977). Use of antibiotics may encourage spread of resistance by other means; for example ampicillin can be shown experimentally to increase transfer of resistance between the Enterobacteriaceae in the intestine, presumably by removal of Bacteroides spp. whose presence inhibits conjugation (Anderson, Gillespie and Richmond, 1973; Anderson, 1975).

There are mechanisms in the bacterial cell that discourage some of these processes. Conjugation in Enterobacteriaceae and Staphylococcus aureus is normally inhibited (Lacey, 1980), and the process of transposition itself can be under repression (Bennett and Richmond, 1976). Studies of the above events in vitro are usually made with cultures devoid of such inhibition to obtain maximum frequencies. In nature, inhibitory processes may dominate; this explains why antibiotic resistance does not immediately appear in a great variety of cultures when a novel agent is introduced. Two aspects of our current understanding of resistance should be stressed. Firstly, the evolutionary mechanisms outlined above are complex, and the actual occurrence or development of resistance to any particular antibiotic is unpredictable. Before accepting that the use of certain antibacterial agents prevents or accelerates development of resistance, extensive clinical evaluation is required. As with mutational resistance, there is still uncertainty as to how relevant in-vitro bacterial manipulations are to natural events, particularly in relation to their frequency. Secondly, because antibiotic resistance is often determined by plasmids and because these elements tend to be lost from cells, resistant bacterial populations can be expected to return towards sensitivity when a particular antibacterial agent is withdrawn. Antibiotic resistance should be considered as potentially reversible.

Trimethoprim resistance in strains selected by the clinical use of the drug or to in-vitro exposure of cultures to the drug

In environments free from antagonists to trimethoprim, MICs of TMP for enterobacteria from clinical material fall into one of three categories: (i) sensitive, at usually less than 8.0 μg/ml; some authors take 1–2 μg/ml as the threshold (see Grey, Hamilton-Miller and Brumfitt, 1979); (ii) low-level resistance at 10–500 μg/ml; and (iii)
high-level resistance 1000 µg/ml or more. During the past 10 years the proportion of strains of enterobacteria with high-level resistance has increased (Fleming, Datta and Gruneberg, 1972; Lacey et al., 1972; Jobanputra and Datta, 1974; Amyes, Emmerson and Smith, 1978; Grey et al., 1979; Huovinen and Toivanen, 1980; Towner et al., 1980). Low-level resistance is not transferable to E. coli recipients, but high-level resistance is transferable from some cultures. In the non-transferable high-level resistant organisms, there is evidence for transposition of the genes conferring resistance from plasmid to chromosome (see below).

Biochemical mechanisms of high-level trimethoprim resistance

Amyes and Smith (1974) showed that the R factor R388 determined high-level TMP resistance through the synthesis of an additional DHFR. The molecular weight of this abnormal enzyme was 35 000 compared with 21 000 for the natural DHFR (Amyes and Smith, 1976) and the abnormal enzyme was 20 000 times less susceptible to TMP than the natural one. The two enzymes also differed in substrate specificity, and the R388 enzyme conferred high-level resistance to the broad-spectrum DHFR inhibitor, aminopterin. Sköld and Widh (1974) found that another R factor (R483) caused two changes in the DHFR: (a) an increased amount of the enzyme was synthesised, and (b) a novel enzyme was produced with reduced affinity for trimethoprim and increased thermolability compared with the wild-type enzyme. These two DHFR variants represent two common naturally occurring types, classified by Pattishall et al. (1977) as types I and II. Type I exemplified by R483 is synthesised in several-fold greater amounts than the natural chromosomal enzyme. For the type-I enzyme, the 50% inhibition constants (150) of TMP, methotrexate and aminopterin are increased several thousandfold over those of the chromosomal enzyme. Type-II enzymes are even more insensitive to TMP (more so than type I by a factor of at least 100). Both enzymes differ from the chromosomal enzyme in their binding of NADPH, folic acid and 2, 4-diamino pyrimidine. The molecular weights of these plasmid-determined enzymes are 32 000–37 000 and that of the normal chromosomal enzyme c. 18 000. Whilst there is some variation in the properties of TMP-sensitive DHFRs amongst different bacterial species (Then and Riggenbach, 1978), there are no TMP-sensitive DHFRs with molecular weights similar to those of the TMP-resistant DHFRs. It is not possible to envisage how the resistant enzyme could have evolved from the normal bacterial enzyme. This has led to the suggestion that the abnormal enzyme is derived from a bacteriophage enzyme (e.g., R388-coded enzyme may have been derived from T4 or T6 phage DNA; Sköld and Widh, 1974). However, the DHFR determined by cells harbouring R483 differs in several important respects from DHFR of the T-even bacteriophages (Mosher, DiRenzo and Matthews, 1977). A third TMP resistant DHFR has also been described (Tennhammer-Ekman and Sköld, 1979).

The amino-acid sequence of Type-II enzyme coded by cells harbouring R67 has been elucidated (Smith et al., 1979; Stone and Smith, 1979). This TMP-resistant DHFR has a molecular weight of ~ 35 000 and contains four identical subunits of mol. wt 8444 consisting of 78 amino-acid residues. Comparison of this sequence with that of all known DHFRs shows no significant homology (Stone and Smith, 1979).
Biochemical mechanisms of low-level trimethoprim resistance

Grey et al. (1979) studied 133 TMP-resistant strains of Enterobacteriaceae. The usual mechanism of the resistance in the 120 cultures showing low-level resistance was an abnormal DHFR with decreased sensitivity to TMP. In only one strain examined was there evidence of greater enzyme activity, and in three decreased permeability might have contributed to the resistance. In contrast, TMP-resistant mutants selected in vitro can often contain increased amounts of essentially normal DHFR (Burchall, 1970; Baccanari et al., 1975; Sheldon and Brenner, 1976). This is another example of the difference between the nature of resistance in clinical strains and in variants selected in vitro. Then and Hermann (1981) have recently examined enterobacteria with low-level resistance to TMP from Turku, Finland. Six cultures were found to produce a TMP-sensitive DHFR in normal or slightly elevated amounts. The relationship of these to DHFRs sensitive to TMP is not known. The cultures were resistant to nalidixic acid, it is not known whether TMP alone, TMP/SMX or nalidixic acid selected their appearance.

Genetic basis of low-level resistance to TMP in clinical strains

Because of the extreme difficulty of selecting recipients with low-level resistance to TMP during genetic transfers, little is known about the genetic basis of low-level resistance. Many authors consider it to be mainly chromosomal; this is likely, but proof is wanting.

Genetic basis of high-level resistance in TMP in clinical strains

High-level transferable TMP resistance was first reported by Fleming et al. (1972). The plasmids involved were of incompatibility group W, as were most other plasmids determining TMP resistance isolated in the UK during the next few years (Datta and Hedges, 1972; Fleming et al., 1972). In subsequent years, TMP resistance has been determined by many different classes of R factor (Towner, 1979, 1981). Three or possibly four different transposons have been identified; these may have been responsible for the migration of the genes coding for high-level TMP resistance between replicons, including the bacterial chromosome.

The best characterised of such transposons, TnC, was detected in 1976 by Datta and her colleagues in R483 (Barth et al., 1976). A piece of DNA (mol. wt c. 9 \times 10^6) carrying genes conferring resistance to streptomycin and TMP could be transposed to other replicons, including the bacterial chromosome from where further migration could occur. As with other transposons, the attachment of TnC was site-specific and occurred independently of the rec A^+ gene. A strain of E. coli with chromosomal high-level TMP resistance isolated from infected urine contains a transposon similar to the above (Towner, 1979) and may have resulted from transposition of the plasmid gene to the chromosome. However, the frequency of transposition in vivo is unpredictable and this is illustrated by the survey of Datta et al. (1979). During a 3-month period, an epidemic of infections with Klebsiella aerogenes K16 was monitored closely in a hospital. Different clones of this strain contained a cryptic (i.e., its function was not apparent) plasmid of mol. wt c. \ (~90 \times 10^6 and a plasmid of mol. wt
65 × 10⁶ of incompatibility group M. Although the latter plasmid was also found in a few environmentally related cultures of *Citrobacter koseri* and *E. coli*, its properties remained constant and notably the region coding for resistance to TMP and streptomycin that resembled transposon C (Datta et al., 1979). In most of the strains highly resistant to TMP studied recently by Richards and Datta (1981), the resistance was determined by a transposon probably similar to TnC. Studies on plasmids of the W incompatibility group, which often determine TMP resistance, have, in contrast, shown a common DNA core with variation around it. Most of the plasmids are of mol. wt 20 × 10⁶ in size with a 13 × 10⁶ segment common to most (Gorai et al., 1979). Variations in the plasmids are seen typically among the antibiotic resistance genes, particularly in the presence of a transposon.

Shapiro and Sporn (1977) have described a transposon (Tn402) coding for TMP resistance, capable of insertion into and excision from phage λ. This transposon arose in plasmid R751 of incompatibility group P126. This is consistent with the proposal that viral DHFR may be responsible for the origin of high-level TMP resistance.

Trimethoprim resistance in *Salmonella* may be determined by a transposon (Richards et al., 1978). Chromosomal high-level resistance to TMP appears to be potentially dangerous because it is associated with permanent alterations of the bacterium's genes. However, two considerations mitigate against this: (1) the transposon could be lost from the cell after migration from the chromosome to a plasmid, if it is assumed that the chromosomal copy is lost upon migration; (2) bacteria containing such an element may be diploid for DHFR or produce greater than usual amounts of a single DHFR. If so, in non-selective environments, i.e., in the absence of TMP, they may be at a slight disadvantage compared with the wild type and hence tend to be overgrown by the latter.

Thus, in summary, the biochemical and genetic mechanisms of naturally occurring resistance to TMP are generally different from those of cultures selected for TMP resistance in vitro. Furthermore, the complexity of naturally occurring TMP resistance is evident. This necessitates reliance on information from clinical trials, rather than from laboratory manipulations, to assess the risks of resistance developing after the use of TMP or TMP/SMX.

**Selection of resistance to TMP in commensal organisms**

Trimethoprim (TMP) usually in combination with sulphamethoxazole (SMX) as co-trimoxazole (Co-T) has been used mainly for treating urinary, respiratory and soft-tissue infections, and the causative organisms of the infections concerned are often derived from the commensal flora. This is particularly true of urinary-tract infections, and selection of resistance in commensal organisms is evidently an important consideration.

There has been a belief for many years that subinhibitory concentrations of antibiotics are prone to select antibiotic resistance in vivo—hence the empirical dogma that antibiotics should be prescribed in high dose as a “complete” course. However, there is no proof that long courses prevent resistance, and there is some evidence to the contrary. For example, some workers have cured urinary-tract infections with a single dose of an antibacterial agent such as amoxycillin, sulphonamides or Co-T, without evidence of rapid development of resistance (Bailey and Abbott, 1977; Källenius and...
Winberg, 1979; Bailey and Blake, 1980). In acute urinary-tract infections in the elderly we have analysed the incidence of TMP resistance in urinary pathogens and in the colon enterobacteria after a single dose of TMP or 10 doses over 5 days. More resistance to TMP was selected with the longer course (Lacey et al., 1981).

Co-trimoxazole is well known to reduce the growth of members of the Enterobacteriaceae in the colon (Moorhouse and Farrell, 1973), the principal reservoir for recurrent urinary-tract infections. Part of the success of Co-T in the prophylaxis of urinary infections (Smellie et al., 1976) can be attributed to this effect. If TMP alone is used similarly it is particularly important to know whether TMP selects resistance in the intestinal enterobacterial population. Toivenan et al. (1976) compared the effect of TMP alone with Co-T administered for 3 weeks to 30 healthy volunteers. Trimethoprim resistance was not associated with either regimen. These findings were confirmed and extended by Knothe (1979) in studies with healthy individuals over 12 weeks. Reduction in the numbers of the Enterobacteriaceae was achieved without selection of resistance. Pancoast, Hyams and Neu (1980) studied the vaginal and faecal flora after TMP or TMP/SMX was given to young women with acute urinary infections. Neither drug regimen selected TMP-resistant bacteria at these sites. More recently, Guerrant et al. (1981) studied the effect of Co-T, TMP 200 mg daily, or TMP 400 mg daily on resistance among the faecal flora. None of these regimens selected TMP-resistant E. coli, but TMP 400 mg daily selected Pseudomonas and some related species.

**Resistance developing during therapy with trimethoprim**

Most patients who have been treated with trimethoprim alone have had acute, recurrent or chronic urinary infections, but chest infections and typhoid fever have been studied recently (table II). If the use of TMP alone had been particularly liable to select resistance in vivo, it should have been evident during use, because most studies have assessed cure bacteriologically on completion of therapy. The cure rates varied from near 100% for acute uncomplicated urinary infections in non-hospital patients to about 50% in patients with recurrent or difficult infections. Only five groups of workers have described TMP resistance in the urinary pathogen after treatment with TMP (Sourander, Saarumaan and Arvilommi, 1972; Kasanen et al., 1974a; Kasanen, Sundquist and Junnila, 1979; Pearson et al., 1979; Lacey et al., 1980a). The pathogens concerned were mainly E. coli and Proteus and comprised about 1% of the cultures. It is not known whether the resistant organism was derived from the original sensitive organism.

In each of the studies comparing TMP with TMP/SMX, there was no report of a higher incidence of TMP resistance after the use of TMP alone. The rarity of TMP resistance after its prophylactic use for periods extending from a month to several years is particularly remarkable (Kasanen et al., 1978; Kunin, Craig and Uehling, 1978; Pearson et al., 1979).

Although the cure rates with TMP are generally very similar to those obtained with the TMP/SMX combination (Brumfitt and Pursell, 1972; Koch et al., 1973; Kasanen et al., 1974b; Kasanen, Sundquist and Junnila, 1979; Lacey et al., 1980a), two studies revealed higher cure rates with the combination than with TMP alone. In one, Sourander et al. (1972) found that TMP produced a cure in only 9 of 19 elderly patients
<table>
<thead>
<tr>
<th>Author</th>
<th>Nature of infection</th>
<th>Trimethoprim treatment schedule</th>
<th>Number of patients cured/ number evaluated (and percentage)</th>
<th>Notes on possible emergence of resistance</th>
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</thead>
<tbody>
<tr>
<td>Schnaars and Escher (1969)</td>
<td>Urinary infection</td>
<td>First day 750 mg, then 500 mg daily for up to 2 weeks</td>
<td>14/21 (67)</td>
<td>No mention</td>
</tr>
<tr>
<td>Hoigne, Müller and Schneider (1969)</td>
<td>Urinary infection acute and chronic</td>
<td>250 mg daily for 7 days (acute) 250 mg for 28 days (chronic) 200 mg daily for 10–14 days</td>
<td>44/47 (94) 48/65 (74) 16/39 (41)</td>
<td>No mention</td>
</tr>
<tr>
<td>Cox and Montgomery (1969)</td>
<td>Recurrent urinary infection with failed sulphonamide treatment</td>
<td>Acute urinary infection and bacteriuria</td>
<td>400 mg daily for 7 days</td>
<td>70/84 (83) Failure of response not attributed to bacterial resistance 3 strains of <em>E. coli</em> and <em>Proteus</em> resistant after therapy</td>
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<tr>
<td>Brumfitt and Pursell (1972)</td>
<td>Geriatric patients with chronic urinary infection due to sulphonamide-resistant bacteria</td>
<td>320 mg daily for 15 days</td>
<td>8/19 (42)</td>
<td>No mention</td>
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<td>Sourander, Saarimaa and Arvilommi (1972)</td>
<td>Chronic urinary infection; 85%, with structural lesions</td>
<td>400 mg daily for 10 days</td>
<td>96/146 (66)</td>
<td>No mention</td>
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<td>Gieckman (1973)</td>
<td>Acute urinary infection</td>
<td>480 mg daily</td>
<td>22/23 (96) 1.5, recurrences per treatment month (267 treatment months)</td>
<td>No mention</td>
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<td>Koch <em>et al.</em> (1973)</td>
<td>Recurrent urinary infection</td>
<td>100 mg daily for 1–8 months</td>
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<td>Kasanen <em>et al.</em> (1974a)</td>
<td>Acute urinary infection</td>
<td>500 mg daily for 10 days</td>
<td>45/51 (88) Three <em>E. coli</em> and <em>Proteus</em> infections persisted; not known whether resistant</td>
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<td>Kasanen <em>et al.</em> (1974b)</td>
<td>Recurrent urinary infection</td>
<td>400 mg daily for 10 days</td>
<td>15/25 (60) No mention</td>
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<tr>
<td>Mannisto (1976)</td>
<td>Urinary infection: 16 acute, 4 recurrent, 10 chronic</td>
<td>200 mg daily for 6 months then 100 mg daily for up to 3 years</td>
<td>No trimethoprim resistant enterobacteria from urine, rectal swab or vagina 3 episodes of infection due to trimethoprim-resistant organisms</td>
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<td>Kunin, Craig and Uehling (1978)</td>
<td>Recurrent urinary infection</td>
<td>100 mg alternate nights “long term”</td>
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<tr>
<td>Study</td>
<td>Condition</td>
<td>Treatment</td>
<td>Outcome</td>
<td></td>
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<td>------------------------------------------</td>
<td>--------------------------</td>
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<tr>
<td>Kasanen et al. (1979)</td>
<td>Urinary infection, mainly acute</td>
<td>320 mg daily for 15 days</td>
<td>131/139 (94)</td>
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<tr>
<td>Stamm et al. (1980)</td>
<td>Prophylaxis of urinary infection</td>
<td>100 mg daily for 6 months</td>
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<tr>
<td>Mabeck and Vejlsgaard (1980)</td>
<td>Bacteriuria in general practice</td>
<td>400 mg daily for 7 days</td>
<td>269/341 (79)</td>
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<td>Lacey et al. (1980a)</td>
<td>Acute chest infection</td>
<td>400 mg daily for 5 days</td>
<td>85/107 (79)</td>
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<tr>
<td>Trimethoprim Study Group (1981)</td>
<td>Acute urinary infection</td>
<td>100–400 mg daily for 7 days</td>
<td>109/111 (98)</td>
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<tr>
<td>McKendrick, Geddes and Farrell (1981)</td>
<td>Typhoid fever</td>
<td>200 mg daily (child), or 600 mg daily for 14 days (7), 28 days (1)</td>
<td>8/8 (100)</td>
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<tr>
<td>Lacey et al. (1981)</td>
<td>Acute urinary infection</td>
<td>200 mg stat (a), or 400 mg daily for 5 days (b)</td>
<td>(a) 33/49 (67) (b) 44/47 (94)</td>
<td></td>
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</tbody>
</table>

Table II (cont.)

One trimethoprim-resistant strain of *E. coli* isolated at end of treatment
No trimethoprim-resistant *E. coli* from urine. One from 316 rectal, urethral or vaginal swabs yielded trimethoprim-resistant *E. coli*
No mention
No resistant *Haemophilus* or pneumococci
No mention
Little trimethoprim resistance acquired by gut flora or bacteria in urinary tract
### TABLE III


<table>
<thead>
<tr>
<th>Author</th>
<th>Organisms tested</th>
<th>Method of sensitivity testing</th>
<th>IP or OP</th>
<th>Number tested</th>
<th>Percentage resistant to trimethoprim</th>
<th>Comments</th>
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</thead>
<tbody>
<tr>
<td>Grüneberg (1972)</td>
<td><em>E. coli</em> from urinary tract &quot;Coliforms&quot; from all sources</td>
<td>Not stated</td>
<td>IP and OP</td>
<td>208</td>
<td>4</td>
<td>...</td>
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<tr>
<td>Lacey et al. (1972)</td>
<td><em>E. coli</em> from urinary tract &quot;Coliforms&quot; from all sources</td>
<td>Disk</td>
<td>IP and OP</td>
<td>725</td>
<td>2</td>
<td>None of the resistance transferable, includes first report of transferable resistance to trimethoprim</td>
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<tr>
<td>Fleming et al. (1972)</td>
<td>Enterobacteria causing urinary infection</td>
<td>Disk</td>
<td>MIC</td>
<td>280</td>
<td>2</td>
<td>Pseudomonas excluded; intermediate zones of inhibition excluded from sensitivity, hence resistance probably overstated</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>1971/1972: 165</td>
<td>13</td>
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<tr>
<td>Williams and Andrews (1974)</td>
<td><em>Haemophilus influenzae</em></td>
<td>MIC</td>
<td>IP and OP</td>
<td>68</td>
<td>0</td>
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<td>Acar et al. (1977)</td>
<td>Enterobacteria from all sources &quot;Coliforms&quot; from all sources</td>
<td>Disk</td>
<td>MIC</td>
<td>16,000 between 1972–1974</td>
<td>17</td>
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<td>Marks et al. (1977)</td>
<td>&quot;Coliforms&quot; from all sources</td>
<td>Disk</td>
<td>MIC</td>
<td>380</td>
<td>13</td>
<td>If a single epidemic trimethoprim resistant strain is excluded, the incidence is 2.4%</td>
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<td>Howard et al. (1978)</td>
<td><em>Haemophilus influenzae</em></td>
<td>Disk</td>
<td>IP and OP</td>
<td>874</td>
<td>0-2</td>
<td>Multi-centre study covering most parts of Great Britain</td>
</tr>
<tr>
<td>McGill (1978)</td>
<td>Urinary enterobacteria</td>
<td>MIC</td>
<td>IP and OP</td>
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<td></td>
<td>58</td>
<td>6</td>
<td>Sensitivity to co-trimoxazole tested; sensitivity to trimethoprim inferred</td>
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<tr>
<td>Kayser and Muller (1978)</td>
<td><em>E. coli</em>. Source not stated</td>
<td>Disk</td>
<td>IP and OP</td>
<td>1974 not stated</td>
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<td>Amyes et al. (1978)</td>
<td>Urinary tract isolates</td>
<td>Disk</td>
<td>IP</td>
<td>1975 604</td>
<td>8</td>
<td>Trimethoprim resistance defined as MIC &gt; 1-0 µg/ml (but for <em>Proteus</em>. &gt; 2-0 µg/ml)</td>
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<td>Grey et al. (1979)</td>
<td>Urinary pathogens</td>
<td>Disk</td>
<td>MIC</td>
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### Table III (cont.)

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<td>Busk and Korner (1980)</td>
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<td>IP and</td>
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<td>OP Klebsiella spp.</td>
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<td>IP Klebsiella spp.</td>
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<td>Richards and Datta (1981)</td>
<td>Urinary E. coli</td>
<td>MIC</td>
<td>OP</td>
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<td>Houvinen, Mäntyjärvi, and Toivanen, 1982</td>
<td>E. coli</td>
<td>MIC</td>
<td>IP (Turku City Hospital)</td>
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<td>IP (Turku University Hospital)</td>
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<td>IP (Kuopio University Hospital)</td>
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</tr>
</tbody>
</table>

IP = specimens from in-patients; OP = specimens from out-patients.
with chronic urinary infection caused by sulphonamide-resistant bacteria. In the other study, Gleckman (1973) showed that TMP was less effective than the combination in treating chronic urinary infection in patients in whom associated structural abnormalities of the urinary tract were common. In these studies the improved clinical response with Co-T is probably attributable to the extended antibacterial spectrum of the combination rather than to suppression of resistance; in particular, in the patients treated with TMP alone, there was a higher proportion of klebsiella infections than in the group treated with Co-T.

**Incidence of trimethoprim resistance during 1971–1981**

The incidence of TMP resistance in common pathogens is shown in table III. A few strains do not appear to have been tested for resistance to TMP alone, and in these resistance to Co-T is assumed to denote resistance to TMP. Although criteria for resistance vary, the following trends emerge: (1) the incidence of resistance amongst the Enterobacteria during 1970–1972 was c. 2–3%. This resistance is typically low-level and non-transferable; (2) there has been a general increase in the incidence of TMP resistance in these organisms during the decade, although marked local variations exist, in the overall incidence and in the prevalence of a particular resistant species; (3) resistance is generally commoner in cultures isolated from hospital patients than from outside; (4) one or two resistant strains, e.g., *Klebsiella* K2 (Acar et al., 1977), have been particularly successful and have caused local "pockets" with a high incidence of resistance; (5) during the decade there has been a trend towards replacement of low-level resistance by high-level resistance.

Two surveys have shown that TMP resistance is rare in *Haemophilus influenzae* and has not increased during 1974–1978 (Williams and Andrews, 1974; Howard, Hince and Williams, 1978). Reports of TMP resistance in *Haemophilus* should be assessed critically because many culture media enriched for *Haemophilus* cultivation contain substances antagonistic to TMP (May and Davies, 1972; Cornere and Menzies, 1974). During therapy with Co-T, the sulphonamide concentration in lung tissue and sputum may be too low to inhibit the growth of *Haemophilus* (Wilkinson and Reeves, 1979). In effect this organism may have been exposed to TMP alone. If TMP did have a particular predilection to select resistance during clinical use, the incidence of TMP-resistant *Haemophilus influenzae* should be high, but it is not.

The question whether the incidence of resistance to TMP would have been different in the enterobacteria had TMP been used singly is difficult to answer. No appropriate long-term prospective study has been performed. Claims that in Finland a high incidence of TMP resistance is causally related to the use of TMP alone (Toivanen and Dornbusch cited by Busk and Corner, 1980) cannot be substantiated from survey data of this type. The overall incidence of resistance fluctuates over the years. Similarly, the nature and control of different epidemic strains vary.

The great majority of TMP-resistant enterobacteria are also resistant to sulphonamides (table IV). This is not surprising because of the high incidence of resistance to sulphonamides (c. 20–40% in cultures from hospital sources) when Co-T was first used. For survival in the presence of Co-T, resistance to both drugs is required. The genes for sulphonamide resistance may be closely linked to those for TMP resistance (Acar et al., 1977) or they may be carried on different replicons (Towner et al., 1980; Richards and
Datta, 1981). The presence of SMX in Co-T has not prevented the appearance of TMP resistance. The use of TMP alone may be expected to select sulphonamide-sensitive TMP-resistant bacteria. The localities where TMP alone replaces the combination may provide opportunities for this to be monitored.

**Trimethoprim-resistant *E. coli* from Helsinki**

We have examined 124 cultures of *E. coli* isolated from urinary-tract infections in Helsinki during 1978 that were found to give reduced zones of inhibition around a disk containing TMP. The resistance was quantified by estimation of MICs on DST agar containing 4% lysed horse-blood, the endpoint being taken as that concentration inhibiting >95% of isolated cells. The results are presented in table V. Only 28% showed high-level resistance, and of these, 31 (89%) were also resistant to sulphonamides (MIC of 50 µg or more per ml). Transferable resistance (to *E. coli* strain J53) was seen in only four highly resistant cultures. There were 37 cultures showing low-level resistance; in none of these was the resistance transferable. Of the four with transferable resistance, TMP resistance was transferred alone from three without sulphonamide resistance, although two of these donor cultures were resistant to sulphonamides. Thus, the properties of TMP-resistant isolates from Helsinki are very similar to those of resistant strains isolated in other countries, although TMP was frequently used alone in Helsinki, but not outside Finland, at the time of these isolations.

**Sensitivity of *E. coli* to trimethoprim and sulphonamides in King’s Lynn Hospitals 1978–1982**

During 1980 and 1981 TMP has been widely prescribed as a single substance for treating urinary-tract and other infections in patients inside and outside hospitals in the King’s Lynn Health District. The figures for hospital prescribing of TMP, sulphonamides and Co-T are shown in table VI. The incidence of TMP resistance was about 7% overall, with that for *E. coli* 5% (table VI). There has been no increase in resistance despite the complete replacement of Co-T with TMP at the end of 1979. Interestingly, the incidence of sulphonamide resistance has declined recently (table VI).
### TABLE V
Trimethoprim resistance in 124 urinary isolates of *E. coli* from Helsinki during 1978

<table>
<thead>
<tr>
<th>Nature of resistance</th>
<th>No TMP resistance or low-level resistance (MIC &lt; 10 μg/ml)</th>
<th>Intermediate levels of TMP resistance (MIC 10-100 μg/ml)</th>
<th>High-level TMP resistance (MIC &gt; 1000 μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number with TMP resistance of stated level</td>
<td>52</td>
<td>37</td>
<td>35</td>
</tr>
<tr>
<td>Number resistant to sulphonamides (MIC 50 μg/ml or more)</td>
<td>9</td>
<td>32</td>
<td>21</td>
</tr>
<tr>
<td>Number with transferable TMP resistance</td>
<td>NT</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Number resistant to sulphonamides (MIC 50 μg/ml or more)</td>
<td>37</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>Number resistant to sulphonamides (MIC 50 μg/ml or more)</td>
<td>52</td>
<td>37</td>
<td>35</td>
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<tr>
<td>Number resistant to sulphonamides (MIC 50 μg/ml or more)</td>
<td>9</td>
<td>32</td>
<td>21</td>
</tr>
<tr>
<td>Number resistant to sulphonamides (MIC 50 μg/ml or more)</td>
<td>NT</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

NT = not tested.

### TABLE VI
Consumption of sulphonamides and trimethoprim and resistance to them in *E. coli* in the hospitals of the King's Lynn Health District

<table>
<thead>
<tr>
<th>Survey period</th>
<th>Total amount of co trimoxazole prescribed</th>
<th>Amount of sulphonamides prescribed as single therapy</th>
<th>Amount of trimethoprim prescribed as single therapy</th>
<th>Number of isolates examined</th>
<th>Percentage number resistant to Sulphonamides</th>
<th>Percentage number resistant to Trimethoprim</th>
</tr>
</thead>
<tbody>
<tr>
<td>1978</td>
<td>2400 g sulphamethoxazole 480 g trimethoprim</td>
<td>1000*</td>
<td>Nil</td>
<td>507</td>
<td>29.5</td>
<td>7.4</td>
</tr>
<tr>
<td>1979</td>
<td>1600 g sulphamethoxazole 320 g trimethoprim</td>
<td>1000*</td>
<td>300</td>
<td>570</td>
<td>29.8</td>
<td>5.1</td>
</tr>
<tr>
<td>Jan.–Aug. 1980</td>
<td>Nil</td>
<td>800</td>
<td>1100</td>
<td>616</td>
<td>21.9</td>
<td>6.5</td>
</tr>
</tbody>
</table>

* Estimated.
Conclusions

Although synergy between TMP and SMX can easily be demonstrated in vitro, there is little evidence that it is a requirement for a therapeutic effect in vivo or is relevant to the prevention of resistance to TMP.

In vitro, the combination of TMP and SMX suppresses the appearance of resistant mutants. However, the properties of the mutants selected in vitro after TMP exposure differ from those isolated from clinical material, except in the case of thymine-requiring cultures. Bacteria passaged in vitro to become highly resistant to TMP contain a cluster of mutations. These cells usually produce increased amounts of unchanged DHFR, whereas high-level resistant cultures from clinical material contain novel DHFRs.

The epidemiology of high-level resistance to TMP follows that of antibiotic resistance in general. Initially a few resistant cultures of the enterobacteria were selected by the use of TMP. Subsequently the gene coding for resistance has spread to other bacteria with its transposition to different replicons including the bacterial chromosome. It is possible that the latter occurs at a frequency higher than that of other resistance mechanisms.

In clinical trials, TMP is as effective as the combination TMP/SMX in the treatment of many infections, and resistant variants rarely emerge during treatment. Resistance to TMP has occurred predominantly in sulphonamide-resistant bacteria. Use of the TMP/SMX combination therefore selects for resistance to both agents. Trimethoprim has replaced co-trimoxazole in the King’s Lynn Health District since 1979 without coincidental development of a high incidence of resistance. More clinical trials, particularly of long-term therapy, are needed to evaluate the extent to which the use of TMP alone may reduce the incidence of sulphonamide resistance and affect the incidence of TMP resistance. When this review was prepared in mid-1982 there was no substantial evidence that TMP used alone will be particularly prone to select resistance.

Any evaluation of data relating to the incidence of resistance must include information on antibiotic usage in general and the measures adopted to contain resistant organisms. As with all antibacterial agents, resistance can be minimised by prescribing TMP only for those patients for whom it is indicated.

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SULPHONAMIDE-TRIMETHOPRIM COMBINATIONS


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SULPHONAMIDE-TRIMETHOPRIM COMBINATIONS


