organism, as judged by increase in generation time caused by concentrations of 2 and 4 mg ml^{-1} whereas *P. mirabilis* F67 (TEM) showed a marked sensitivity to this agent, cell lysis occurring at a concentration of 2 mg ml^{-1}.

Evidence that R-factor DNA is associated with a cellular component, presumed to be the cytoplasmic membrane has been presented (Hershfield, LeBlanc & Falkow, 1973). In an attempt to elucidate the nature of the R-factor induced effect, spheroplasts were produced by treating exponential cells in DM medium containing 0·4 M sucrose with benzylpenicillin (1000 units ml^{-1}). Spheroplast formation, as judged by phase contrast microscopy was virtually complete after 4 h. The sensitivity of the spheroplasts from each strain to lysis by sodium desoxycholate was similar, suggesting that the R-factors have not caused a gross change in the cytoplasmic membrane and furthermore that the different response of whole cells to sodium desoxycholate is associated with modification to a cell envelope component external to the cytoplasmic membrane resulting in an increased permeability.

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R-factor mediated resistance to ultraviolet light

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R-factor 1818 (R-1818) as well as conferring antibiotic resistance, also protects bacteria from ultraviolet irradiation (Drabble & Stocker, 1968). We report experiments that investigate the mechanism of UV-resistance conferred by this R-factor.

Escherichia coli strains were grown and irradiated as described by Tweats, Pinney & Smith (1974).

We found that the R-factor protected *pol* A1 and *lig* mutants of *E. coli*, which are believed to be deficient in the excision repair of ultraviolet-induced damage (Monk, Peacy & Gross, 1971; Howard-Flanders, 1968). This repair pathway can be inhibited in wild-type strains by substituting bromouracil for chromosomal thymine (Hanawalt, 1968). When excision repair was inhibited in this way it was found that the R-factor still conferred ultraviolet protection. These results indicate that the ultraviolet protection conferred by R-1818 is independent of host-mediated excisions repair.

Apart from excision repair, *E. coli* can repair ultraviolet induced damage by recombination repair, which is mediated by the *rec*A, *rec*B and *rec*C genes (Howard-Flanders, 1968). The *rec*A gene product appears to have several functions, including the control of nuclease synthesis by the *rec*B, C genes (Clark, 1974). It was found that R-1818 increased the ultraviolet sensitivity of *rec*A mutants, but still protected *rec*B mutants. This indicates that the plasmid mechanism of ultraviolet protection can act independently of host cell recombination repair.

Bridges (1972) proposed a third repair pathway which he termed reinitiation recovery. He suggested that "UV-stalled" DNA replication forks are removed by enzymes of both the recombination and excision repair pathways, and that chromosome replication is then reinitiated at the origin. Treatment of cells with chloramphenicol (Cm) *before* ultraviolet irradiation, allows chromosome replication to go to completion, but prevents initiation of new rounds of replication (review, Hanawalt, 1968). As no replication forks are present in the DNA of such cells, reinitiation recovery is irrelevant to their survival. We found that R-1818 protected cells treated with Cm *before* ultraviolet-irradiation, and conclude that R-1818 mediated repair can act independently of reinitiation recovery. On the other hand, when bacteria were treated with Cm *after* ultraviolet irradiation R-factor protection was abolished. This finding suggests that the R-factor mediates ultraviolet protection via a protein, which is synthesized *after* the cell is damaged by ultraviolet irradiation.

Our results indicate that the mechanisms of R-1818 mediated ultraviolet resistance is not involved in excision repair or in reinitiation recovery. It appears to be mediated by an inducible gene product, dependent on a functional host *rec*A gene, but which can act independently of recombination repair.

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Trimethoprim resistance in *Escherichia coli*

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Trimethoprim (TM) is a synthetic antimicrobial compound which is administered clinically in combination with sulphamethoxazole as Septrin (Burroughs Wellcome) or Bactrim (Roche). TM is an inhibitor of dihydrofolate (FH_2) reductase with a marked affinity for bacterial enzyme when compared to mammalian enzyme (Burchall & Hitchings, 1965). Bacteria resistant to this and other anti-folates have been isolated, most of them either synthesize more enzyme or synthesize enzyme with altered properties (Burchall, 1970; Albrecht, Palmer & Hutchison, 1966; Sirotnak, Donati & Hutchison, 1964).

Mutants of *E. coli* K12 resistant to high levels (up to 1024 $\mu g\ ml^{-1}$) of TM were isolated by serial subculture in minimal salts medium containing increasing concentrations of TM. The genetic properties of these mutants are being reported elsewhere.

The biochemical properties of FH_2 reductase from a series of TM-resistant mutants were investigated; the protein fractions obtained by precipitation of sonicates with 55 to 90% $(NH_4)_2SO_4$ saturation were assayed by the method of Burchall & Hitchings (1965).

The series showed a progressive increase in specific activity of FH_2 reductase which could be related in part to an increase in V_{max}, as revealed by kinetic analysis, and in part to increased enzyme synthesis, demonstrable as an increase in methotrexate binding. The enzyme from the most resistant strains also differs from that of the wild-type in its response to heat, urea and TM. The response to TM is most surprising in that the enzyme appears to have become more sensitive.

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