A Davis differential cathode ray polarograph was used as a single cell instrument in the direct mode with a start potential of −0·35V. Determinations were made at 25° using the mercury pool anode, and all solutions to be analysed were flushed (5 min) with nitrogen previously passed through support electrolyte.

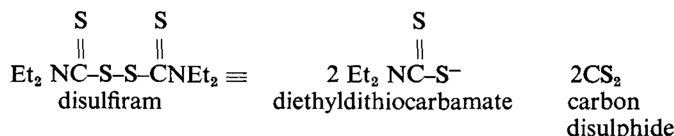
Method: (a) Whole blood (1·0 cm^3) from DS treated patients was heated (boiling water bath) with 50% sulphuric acid (10 cm^3) in suitable apparatus. Liberated CS_2 was swept with a stream of nitrogen into 5·0 cm^3 of diethylamine (2%) in ethanol (95%). After 15 min 0·3% copper nitrate solution (0·1 cm^3) and 0·6 M hydrochloric acid (2·0 cm^3) were added to the diethylamine solution which was then polarographed.

(b) CS_2 in expired air (10–15 litres) was passed via a respirometer through diethylamine solution (5·0 cm^3) and polarographed after treatment with cupric copper and hydrochloric acid as above.

Calibration: (i) DS (1·0 μg–30·0 μg) was included in the diethylamine solution and the determination completed after adding cupric copper and hydrochloric acid.

(ii) citrated whole human blood (1·0 cm^3) loaded with DS (1·0 μg–30·0 μg) was treated as in Method (a).

Equivalence:



Results: Calibration (peak height v weight DS) was linear, 1·0 μg DS = 170 graticule units at maximum sensitivity. Recoveries from blood were 85% to 105% (1·0 μg level) and 95% to 105% (30·0 μg level). Typical results for patients given a single 200 mg dose of DS were as follows:

Time (h) after dose	DS concentration ($\mu g\ cm^{-3}$)	CS_2 concentration ($mg\ m^{-3}$)
6·0	1·4	0·6
12·0	4·5	2·3
18·0	3·0	4·2
24·0	2·3	4·7

The method is in current use for decomposition studies, cell/plasma DS ratio determination and evaluation of new intramuscular DS preparations.

REFERENCE

PORTER, G. S. & WILLIAMS, A. E. (1972). *J. Pharm. Pharmac.*, **24**, *Suppl.*, 144P–145P.

Sensitivity of R^+ strains of *Proteus mirabilis* to sodium desoxycholate

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The ability of *Proteus mirabilis* to swarm on solid media is notorious. During a study of R-factor mediated antibiotic resistance in this organism, it was found that introduction of R-factor TEM (conferring resistance to ampicillin and streptomycin) into *P. morabilis* F67 abolished swarming on solidified Davis-Mingioli (DM) medium. On the other hand, R-factor 1818 (conferring resistance to ampicillin, streptomycin, sulphonamide and tetracycline) enhanced the swarming ability of the organism on this medium. Since swarming is associated with the formation of elongated cells as a result of the inhibition of cell division (Jones & Park 1967), the R-factors may be affecting the surface layers of the cell. Accordingly, exponential cultures in DM medium were challenged with the anionic detergent sodium desoxycholate. *P. mirabilis* F67 (1818) was slightly more sensitive than the parent

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.

REFERENCES

HERSHFIELD, V., LEBLANC, D. J. & FALKOW, S. (1973). *J. Bact.*, **115**, 1208–1211.
JONES, H. E. & PARK, R. W. A. (1967). *J. gen. Microbiol.*, **47**, 359–367.

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.