Strains 57 and 13 can be transduced to streptomycin resistance by phages 12/57 and 34/13 propagated on streptomycin-resistant mutants of 57 and 13 respectively[6]. Lysogenic transductants possess all the properties of strains lysogenized with non-transducing phage.

Differences in properties of strains 57 and 57(12/57) and strains 13 and 13(34/13) are attributed to the presence of converting prophages 12/57 and 34/13. Conversion is in respect of phage-adsorbing capacity and possibly other antigenic changes as well.

Phages 12/57 and 34/13 are thus competent in transducing and converting systems. A similar position obtains with *Salmonella* phage *P22*. This phage has been extensively used in transduction experiments and can produce lysogenic conversion[4]. A possible difference is that this phage is capable of producing conversion in the vegetative state (phage conversion). No attempt has yet been made to investigate the latter possibility for the *Proteus* phages mentioned.

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## Elimination of Transmissible Drug-resistance by Treatment with Acriflavin

THE first isolation of multiple resistant *Shigella* was reported by Kitamoto[1]. This was resistant to four drugs ($r_4$): streptomycin, tetracycline, chloramphenicol and sulphanilamide. *E. coli* $r_4$ was isolated in the epidemic of *Sh. flexneri* 3*a* $r_4$ (refs. 2 and 3). *Sh. flexneri* 2*a* $r_3$ and *E. coli* $r_3$ were also isolated in another epidemic, which were resistant to streptomycin, chloramphenicol and sulphanilamide[4]. *Citrobacter* $r_4$ and *E. coli* $r_4$ were isolated from a dysenteric patient after treatment with chloramphenicol[5].

From the epidemiological investigation of the multiple resistant *Escherichia* in the fæces of human beings[6], we have learned more about the multiple resistant *Shigella* and the general phenomena among Enterobacteriaceae.

Ochiai[7] and Akiba[8] reported that the multiple resistance was transmitted between *Shigella* and *E. coli* following mixed cultivation. This transmission is not mediated by transduction, transformation or filtrable agent, but by cell-to-cell contact[9,10] without regard to the polarity of *F* factor[11]. It has also been shown that the multiple resistance is transmitted between every genera of Enterobacteriaceae[12], whereas perhaps only 3–5 per cent of the strains of *Escherichia* would have given positive results in bacterial recombination.

Artificial elimination of the transmissible drug-resistance by treatment with acriflavin was carried out by Hirota's method[13].

The drug-resistant cells were inoculated into Difco brain-heart infusion broth containing sub-lethal concentrations of acriflavin (20 μgm./ml. for *E. coli* and 10 μgm./ml. for *Shigella*). After overnight incubation at 37° C., the cells were streaked on Drigalski's medium and the drug-resistance of each colony was tested. Heart infusion agar was used for assaying resistance to streptomycin, chloramphenicol and tetracycline. For assaying sulphanilamide resistance, semi-synthetic medium was used. It consists of 1 litre of medium *A* (ref. 14) agar enriched with 2·0 gm. casamino-acid, 10 mgm. tryptophan, 2 mgm. nicotinic acid, 10 mgm. thiamin hydrochloride and 2 gm. glucose. The results are shown in Table 1.

Table 1. FREQUENCY OF ELIMINATION OF DRUG-RESISTANCE BY TREATMENT WITH ACRIFLAVIN

| Micro-organisms | With acriflavin treatment | Without acriflavin treatment |
|---|---|---|
| *E. coli* O-26 $r_4$ | 5/579 (0·9 %) | 0/1,589* |
| ,, $r_3$ | 15/547 (2·9 %) | 0/881 |
| ,, $r_1$ | 0/579 | 0/364 |
| *Sh. flexneri* 3*a* $r_4$ | 241/517 (46·6%) | 0/1,198 |
| ,, $r_3$ | 0/375 | 0/1,177 |
| ,, $r_1$ | 201/583 (36·2 %) | 0/1,116 |

$r_4$, resistant to streptomycin, chloramphenicol, tetracycline and sulphanilamide; $r_3$, resistant to streptomycin, tetracycline and sulphanilamide; $r_1$, resistant to tetracycline.
* Numerator, number of the drug-sensitive colony; denominator, number of the total colonies tested.

The drug-resistance of both *Escherichia* and *Shigella* were eliminated from the drug-resistant cells after treatment with acriflavin, and the frequency of elimination of the drug-resistance was higher in *Shigella* than in *Escherichia*. This fact is in agreement with the finding that the loss of the drug-resistance with *Shigella* was more frequent than with *Escherichia* after 6 months storage in cooked meat media. It was also found that all markers for drug-resistance were lost by treatment with acriflavin and the cells became sensitive to all drugs. The drug-sensitive cells thus obtained were not able to transmit the drug-resistance by mixed cultivation with other drug-sensitive cells.

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