inactivation on substrates of *Brucella melitensis*, *B. abortus* and *B. suis*. Only phage 3 (Tbilisi) showed weaker inactivation on all three substrates of *Brucella*. Our data on the characteristics of the antigenic-serological properties of these twenty brucellaphages[2] have shown that phage 3 undergoes the weakest neutralization under the influence of antiphage sera.

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Linked Transduction in *Proteus mirabilis*

Linked transduction in *Proteus* has not hitherto been described. This investigation was carried out to determine possible linkage between arginine loci and other gene loci in *Proteus mirabilis* strain 13 (ref. 1).

Argininoless auxotrophs were selected after treatment with manganese chloride and hydrogen peroxide with a penicillin enrichment technique[2] and replication to minimal medium[2] containing nicotinic acid (0·006 g/l.). The growth requirements of mutants were determined auxanographically. The arginine mutants were then subjected again to the same treatment. A total of 111 arginine mutants, with various additional growth requirements, was finally obtained. Replication in this case was to minimal medium supplemented with arginine hydrochloride (15 μg/ml.).

High titre stocks of phage $\frac{34}{13}$ were prepared, sterilized and treated with ultra-violet light as previously described[2]. Transduction experiments were carried out with an input phage multiplicity of five. After an absorption period of 1 h at 37° C, the tubes were centrifuged, deposits resuspended in saline and portions were plated out. Selection was made on minimal medium and medium supplemented with 10 μg/ml. of the appropriate growth factors. Plates were incubated at 37° C for 72 h. Recipient controls had the phage lysate replaced by (*a*) broth and (*b*) a phage lysate of the recipient organism. The growth requirements of transductant clones were determined by re-streaking or replicating to minimal medium. At suitable intervals, all colonies were picked off into broth for auxanography.

Phage suspensions were always sterile. Two of the double mutants examined could be transduced to prototrophy with phage lysates prepared on the wild-type organism (Table 1). One is mutant requiring arginine and uracil, AC_3Ur, the growth requirements of which are met by arginine or citrulline (but not ornithine, argininosuccinate or carbamyl phosphate) and uracil. Other pyrimidines could not be substituted for uracil. The other mutant, AC_1Me, required the same growth factors from the arginine pathway together with methionine or homocysteine. The mutations in these two double auxotrophic strains were independently selected. To verify that the uracil and methionine second mutations in the mutants AC_3Ur and AC_1Me respectively are indeed separate lesions, phage lysates of the parent arginine requiring

Table 1

Donor	Recipient	No. transductant colonies	Transductants (%) $AC_3^+Ur^+$	$AC_3^+Ur^-$	$AC_3^-Ur^+$
13 (wild)	AC_3Ur	2740	66·6	11·6	21·8
AC_3	AC_3Ur	558	0	0	100
AC_3Ur	AC_3Ur	0	0	0	0
			$AC_1^+Me^+$	$AC_1^+Me^-$	$AC_1^-Me^+$
13 (*wild*)	AC_1Me	7980	0·6	73	26·4
AC_1	AC_1Me	520	0	0	100
AC_1Me	AC_1Me	0	0	0	0

Recipients were infected with a phage input multiplicity of 5. After an adsorption period of 1 h at 37° C, tubes were centrifuged, deposits resuspended and aliquots plated on selective media. Clones were examined by restreaking, replica plating or auxanography.

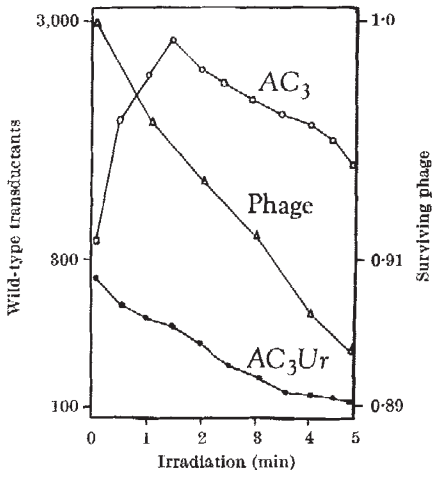

Fig. 1. Effect of ultra-violet irradiation of phage lysates on single and linked transduction. Portions of phage lysates of wild-type organism were irradiated in the dark and then used in transduction experiments with the single and double auxotrophic mutants AC_3 and AC_3Ur, respectively, as recipients. Selection was made on minimal medium. ○, Wild-type transductants with mutant AC_3 as recipient; ●, wild-type transductants with double auxotroph AC_3Ur as recipient; △, plaque-forming units

auxotrophs were also used in transduction experiments with these double auxotrophs as recipients (Table 1). Wild-type colonies were not encountered on control plates.

The transduction rate of AC_3Ur to prototrophy is high and implies close linkage of the particular arginine and uracil markers. This linkage is interesting in view of the biochemical and control relationships of the arginine and pyrimidine pathways[3]. Linkage between arginine and methionine loci in *E. coli* has been described[4]. The results presented here reveal distant linkage between the arginine and methionine loci studied.

The differential effect of ultra-violet irradiation of phage on linked and single transduction is shown in Fig. 1. These findings agree with those of Holloway *et al.*[5].

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Fixed *L*-forms of *Agrobacterium tumefaciens* induced by Ultra-violet Light Radiation

Reversible and fixed *L*-forms of *Agrobacterium tumefaciens* have been obtained using glycine as inducing agent, and also with penicillin[1,2]. The characteristics of the bacterial strain play a definite part in the formation of spheroplasts. In *A. tumefaciens* this property is extremely important. The *A.T.* strain used by Rubio-Huertos and Beltrá readily formed spheroplasts which multiplied for years, even in media lacking glycine, without reverting to the normal bacterial form. By contrast, three other strains in the same conditions failed to do so.

Challice and Gorril[3] observed the formation of filaments and spherical ghosts on *Escherichia coli* after induction with ultra-violet light. Mandel *et al.*[4] have grown *Proteus P18 bacillus* in an ordinary medium containing 20 per cent horse serum and varying amounts of radioactive phosphorus and have observed the formation of long filaments which became globular forms after 21 h in a