blue (a thiazine dye), and riboflavine, are as efficient sensitizers for the inactivation of transforming principle as they are for the photosensitized oxidation of *p*-toluenediamine. Contrary to our previous report[2], we have since found that in the strict absence of oxygen, and using ethylenediaminetetraacetic acid as electron donor, neutral red (an azine dye) can be photoreduced. In the presence of no more than a trace of oxygen it can sensitize the photopolymerization of acrylamide. Our failure to control the oxygen level accounts for the difference between the present results and those obtained earlier. Spectrophotometric studies also show that neutral red is a sensitizer for the photodynamic oxidation of *p*-toluenediamine. In conformity with these findings the present study shows that neutral red is also able to act as sensitizer for the photodynamic inactivation of transforming principle.

Thus, in every case the ability of the dyes to photosensitize the autoxidation of the organic substrate is paralelled by their ability or inability to sensitize the inactivation of transforming principle.

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The isolation of bacterial mutants defective in amino acid transport

Many bacterial strains accumulate amino acids from the surrounding medium[1–4]. The mechanism is obscure, but the phenomenon clear. SCHWARTZ, MAAS AND SIMON[5] have reported that mutants of *Escherichia coli* W with an impaired concentrating mechanism for arginine can be isolated by selection on canavanine-supplemented plates, and for glycine by selection on D-serine-supplemented plates.

We have successfully used another method for isolation, and have obtained three mutant types of *E. coli* W which are defective in the ability to accumulate either histidine, proline or glycine. The first two are new; the last one presumably similar to that isolated by SCHWARTZ *et al.*[5].

For simplicity, these mutants are referred to below as transport-negative (Tr^-). These mutants may be analogous to the permease mutants of the β-galactoside system[4,6], but we prefer the general term "transport" because the process is not understood.

The isolation procedure starts with the use of an auxotroph, *e.g.*, His^-, deficient in some step in histidine biosynthesis, but capable of accumulating histidine from the medium. The wild type (with respect to the transport character), hence $His^-Tr^+_{his}$,

grows well on media or plates containing as little as 10 μg/ml L-histidine, although the final concentration of bacteria reached after growth, or the colony size on plates, is limited. The mutant, His$^-$Tr$^-_{his}$, will grow rapidly only when large amounts—greater than 250 μg/ml—are supplied. At high levels, the supplement presumably diffuses in at a sufficient rate so that the rate of growth is no longer limited by the size of the internal pool of required nutrient.

An overnight culture of a His$^-$Tr$^-_{his}$ auxotroph of *E. coli* W was irradiated with u.v. light to about 0.1 % survival. 5 ml of the irradiated culture were grown in 50 ml of minimal medium A (see ref. 7) supplemented with 1 mg/ml L-histidine. After overnight growth, the cells were centrifuged, washed in buffer, and diluted in 15 ml of A plus 10 μg/ml histidine. The culture was shaken at 37° for 1 h, penicillin added (final concn., 2000 units/ml), and the incubation continued. To follow the time course of killing, three serial samples, appropriately diluted, were spread on nutrient plates at approximately hourly intervals, according to the modified penicillin selection method of GORINI AND KAUFMAN[8].

At these same intervals, about 5 ml of the culture were passed through an HA-Millipore filter* (pore size 0.45 μ), and washed with buffer. The filter, with adherent bacteria, was pushed into a tube containing medium A plus 1000 μg/ml histidine. After growth overnight, the bacterial culture in the tube corresponding to the sample which showed considerable killing by penicillin, was treated further. The cells were centrifuged, washed, and treated exactly as the day before. After two cycles of selection in penicillin and regrowth, appropriate dilutions were spread on plates containing 1 mg/ml histidine, with subsequent replica plating[9] on 10 μg/ml plates. On 1 mg plates, His$^-$Tr$^+_{his}$ and His$^-$Tr$^-_{his}$ colonies were similar in size; on 10 μg plates His$^-$Tr$^+_{his}$ grew to small-sized colonies, but His$^-$Tr$^-_{his}$ colonies were invisible to the naked eye.

Similar procedures with minor modifications were used to isolate the other mutants. The glycine transport-negative mutants were found readily; after one cycle of penicillin treatment, more than 25 % of the survivors were Gly$^-$Tr$^-_{gly}$. This frequency is consistent with the large number of mutants in *E. coli* W resistant to D-serine, as described by DAVIS AND MAAS[10]. Selection methods using D-serine, which readily produce transport-negative mutants (Tr$^-_{gly}$) of *E. coli* W fail for *E. coli* B, which is not sensitive to this inhibitor. Gly$^-$Tr$^-_{gly}$ mutants of *E. coli* B, however, were easily isolated by the penicillin method. For isolation of His$^-$Tr$^-_{his}$ and Pro$^-$Tr$^-_{pro}$, two or three cycles of treatment with penicillin were necessary, and only a few were found among several hundred colonies examined.

A reconstruction experiment, using His$^-$Tr$^+_{his}$ S^s and His$^-$Tr$^-_{his}$ S^r (S^s = streptomycin sensitive; S^r = streptomycin resistant) showed that the selection technique did not produce significant death of the Tr$^-_{his}$ mutant during treatment with penicillin. All Tr$^-_{his}$ mutants isolated have shown measurable reversion rates to Tr$^+_{his}$. Reversion from Tr$^-_{his}$ to Tr$^+_{his}$ is independent of reversion from the auxotroph to prototroph state. When colonies on plates or slants containing 500 to 1000 μg/ml of supplement are tested after many days of storage at 5–10°, the proportion of Tr$^+_{his}$ and His$^+$ in an originally pure His$^-$Tr$^-_{his}$ colony rises. This may be due to depletion of supplement in the agar which surrounds the colony, resulting in a growth advantage for Tr$^+_{his}$ or

* Millipore Filter Corporation, Bedford, Mass. (U.S.A.).

His^+ revertants. Colonies on slants need frequent retesting, and storage in frozen form with 5 to 10 % glycerol is preferred.

Studies of the rate of uptake of ^{14}C-labeled amino acids show that the defect is selective. Cultures were grown to the exponential phase in a medium containing 500 μg/ml of the required amino acid. Chloramphenicol was added (final concn. 200 μg/ml) and the cells ($5 \cdot 10^8$–10^9/ml) incubated for 30 min. The cells were then washed, resuspended in medium A + 50 μg/ml chloramphenicol and reincubated for an additional 30 min. The specified [^{14}C]amino acid was added, and incubation continued for 5 min. An aliquot was withdrawn, filtered through an HA-Millipore filter, and the radioactivity in the washed bacteria measured with a Nuclear-Chicago thin-window counter. The figures in Table I are corrected for the radioactivity which remains in the cellular residue after 5 min at 100°. This correction amounts to at most 5 % of the observed count for both Tr^+ and Tr^- mutants.

The difference in histidine content of Tr^+_{his} and Tr^-_{his} mutants can be seen with 10 μg/ml [^{14}C]histidine in the medium (Table I). The disparity is accentuated when

TABLE I

ACCUMULATION OF RADIOACTIVE AMINO ACIDS BY TRANSPORT-POSITIVE AND TRANSPORT-NEGATIVE AUXOTROPHS IN *Escherichia coli* W.

Bacterial population about 10^9 cells/ml. Medium contained 50 μg/ml chloramphenicol. L-proline and L-histidine were randomly labeled. Glycine, DL-phenylalanine and DL-lysine were labeled in the C-2 position. Samples were taken 5 min after addition of radioactive amino acid. Only one amino acid was added to any tube, and experiments on each pair of mutants were run in parallel. Data assume complete equilibration of [^{12}C]- and [^{14}C]amino acids.

Mutant	*Concentration of amino acid in medium in μg/ml*	*(μg/10^{12} cells)*				
		Glycine	*Proline*	*Phenylalanine*	*Histidine*	*Lysine*
$His^-Tr^+_{his}$	10	140	160	135	600	400
$His^-Tr^-_{his}$	10	250	200	90	92	360
$His^-Tr^+_{his}$	1	—	—	—	540	—
$His^-Tr^-_{his}$	1	—	—	—	15	—
$Gly^-Tr^+_{gly}$	10	360	225	160	500	140
$Gly^-Tr^-_{gly}$	10	40	250	140	500	145
$Gly^-Tr^+_{gly}$	1	400	—	—	—	—
$Gly^-Tr^-_{gly}$	1	10	—	—	—	—
$Pro^-Tr^+_{pro}$	10	400	176	400	500	370
$Pro^-Tr^-_{pro}$	10	600	64	320	310	260
$Pro^-Tr^+_{pro}$	1	—	165	—	—	—
$Pro^-Tr^-_{pro}$	1	—	15	—	—	—

only 1 μg/ml is present, reaching a ratio for Tr^+_{his}/Tr^-_{his} of about 35. The expected distribution of L-histidine due to passive diffusion along electrochemical gradients cannot be calculated with confidence because several physical parameters are unknown, *e.g.*, the intracellular water space of the bacterium and the difference in electrical potential between intracellular and extracellular fluids. The quantity of histidine in Tr^-_{his}, however, is close to that predicted by the assumption of passive distribution. The physical state of the amino acid accumulated by the Tr^+_{his} mutants is open to conjecture. Preliminary experiments with intact[11] and lysed protoplasts of Gly^- Tr^+_{gly} and $Gly^-Tr^-_{gly}$ show that the cell fraction which includes cell membrane is partly responsible for the transport specificity.

Growth curves in liquid media for $His^-Tr^+_{his}$ and $His^-Tr^-_{his}$ are identical when

the histidine level is above 250 μg/ml. At 100 μg/ml, marked slowing of $His^-Tr^-_{his}$ growth appears. At 10 μg/ml the population size of $His^-Tr^-_{his}$ is stationary while $His^-Tr^+_{his}$ grows rapidly. Assay of the medium before and after incubation with $His^-Tr^-_{his}$ showed no destruction of histidine by the bacterial culture. Similar results were obtained with the proline and glycine mutants.

Two other mutants have also been isolated by the method described above—the first is a mutant of *E. coli* W which, unlike the phenylalanine auxotroph from which it was derived, requires very high supplements of phenylalanine for growth. This mutant, however, accumulated ^{14}C-labeled phenylalanine as effectively as the wild-type, and the reason for the very high requirement for phenylalanine is not known. A second mutant (derived from an auxotroph of *E. coli* B requiring histidine, methionine and leucine) is defective in potassium transport, as shown by studies of growth in media containing low potassium (10^{-4} *M*) and measurement of accumulation of ^{42}K (see ref. 12).

The specificity of the defect in these mutants has not yet been fully determined. Complete characterization awaits further isolation of transport-negative mutants.

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Intermittent ultrasonic disruption and localisation of enzymes in acetic acid bacteria

Ultramicroscopic particles, containing a great variety of enzymes, mostly oxidases, can be isolated from disrupted acetic acid bacteria[1–5]. Previously we brought evidence in favour of the view that these enzyme-bearing particles do not exist as such in the

Abbreviations: TPN, triphosphopyridine nucleotide; Tris, tris(hydroxymethyl)aminomethane.