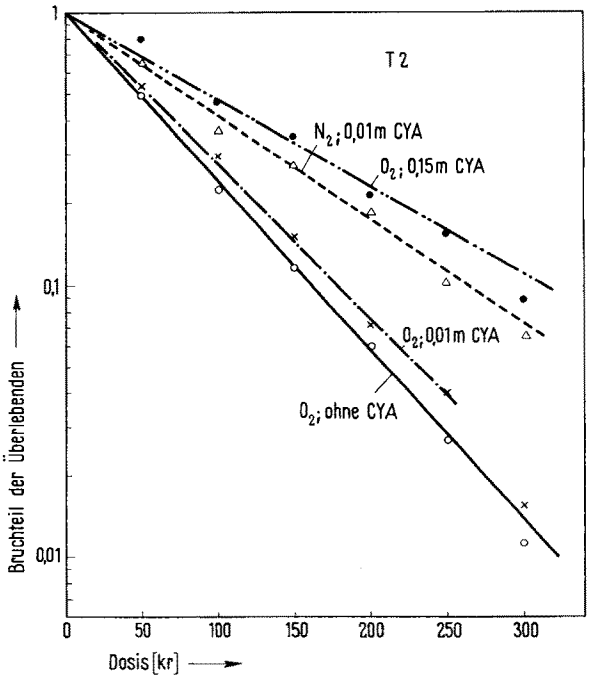
Versuch 1:10 in Difco Nährbouillon (4%) ohne bzw. mit einem Zusatz von Cysteamin (Fa. Fluka, Schweiz) bei pH 7 verdünnt. Bei Bestrahlung in schutzstoffhaltigen Suspensionen liessen wir diese vor Bestrahlungsbeginn 10 min bei Zimmertemperatur auf die Phagen einwirken. Zur Bestrahlung wurden 0,6 ml der Phagensuspension in Plexiglasschälchen von 20 mm Durchmesser abgefüllt. Der mehrfach beschriebene Einfluss des Sauerstoffs auf die Strahlenempfindlichkeit cysteamingeschützter T2-Phagen[4-6] machte es erforderlich, unter kontrollierten Gasbedingungen zu bestrahlen. Mittels eines kleinen Gebläses wurde die Suspension vor und während der Bestrahlung durchmischt und auf diese Weise gleichzeitig mit Sauerstoff oder gereinigtem Stickstoff äquilibriert. Eine Röntgenröhre mit Berylliumfenster von 1,5 mm Stärke gab bei 100 kV_s und 25 mA am Ort der Suspension eine Dosisleistung von 500 kr/min bei einer effektiven Wellenlänge von 1,4 Å. Die in der Phagensuspension absorbierte Strahlenenergie wurde in der gleichen Geometrie mit dem Fricke-Aktinometer ($Fe^{++} \rightarrow Fe^{+++}$) gemessen. Als Kriterium des Strahleneffektes wurde die Plaquebildungsfähigkeit gewählt.



Überlebensrate von T2-Bakteriophagen in 4% Difco-Nährbouillon nach Röntgenbestrahlung unter verschiedenen Bedingungen.

Die Figur veranschaulicht den Einfluss von Sauerstoff während der Röntgenbestrahlung auf die Neigung der Dosis-Effektkurve bei in Cysteaminbouillon suspendierten Phagen T2. Alle experimentellen Daten lassen sich im halblogarithmischen Raster durch Gerade darstellen. Die Neigungsdifferenz der Geraden für Bestrahlung *ohne* Cysteamin und mit 0,01 m Cysteamin (beide Versuche unter O_2) liegt innerhalb der Fehlergrenzen, das heisst, bei Anwesenheit von Sauerstoff bewirkt 0,01 m Cysteamin keinen Schutz. Unter Stickstoff (das ist bei Ausschluss von Sauerstoff) liefert Zusatz von m/100 Cysteamin zur Bouillon eine Schutzwirkung entsprechend einem Dosisreduktionsfaktor von 1,6[8]. Praktisch der gleiche Schutzeffekt ergibt sich auch unter Sauerstoff nach Erhöhen der Cysteaminkonzentration auf 0,15 m; die Neigungen der entsprechenden Kurven unterscheiden sich um weniger als 10%.

Wendet man den von HOWARD-FLANDERS für Bakteriophagen vorgeschlagenen Konkurrenzmechanismus zwischen Sauerstoff und Wasserstoff-Donatoren in Form der Schutzmoleküle um strahleninduzierte freie Radikale in den Phagen auf unsere Ergebnisse an, so ergeben sich zwanglos folgende Schlüsse: Bei Zugabe von Cysteamin zur Bestrahlungsbouillon in Konzentrationen von $\leq$ m/100 gelingt es den Wasserstoff-Donatoren nicht, in Konkurrenz mit den Sauerstoffmolekülen zu treten, die in einer O_2-gesättigten Bouillon überwiegen. Wird dagegen die Cysteaminkonzentration um etwa eine Grössenordnung erhöht, so ist das Gleichgewicht zugunsten der Wasserstoff-Donatoren verschoben, und es kommt zur Reparierung der etwa als strahleninduzierte Carbonium-Radikale angenommenen latenten Strahlenschäden. Abgesehen von der Aufklärung der oben erwähnten scheinbaren Widersprüche, die beim Vergleich von Versuchsergebnissen aus verschiedenen Arbeitskreisen auftraten, scheinen die hier mitgeteilten Ergebnisse geeignet zu sein, das Verständnis für den Schutzmechanismus sulfhydrylhaltiger Verbindungen bei suspensierten röntgenbestrahlten Bakteriophagen zu vertiefen.

Summary. T2 phage suspended in broth and irradiated in the presence of cysteamine in high concentration is protected against the action of X-rays equally well under oxygen as under nitrogen. Low concentrations of cysteamine, however, give protection under nitrogen only. These results resolve apparant contradictions in earlier work and lend support to the mechanism of reaction as proposed by HOWARD-FLANDERS.

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Institut für Strahlenbiologie am Kernforschungszentrum Karlsruhe (Deutschland), 10. Juli 1961.

[8] G. HOTZ und A. MÜLLER, Z. Naturforschg., im Druck.

The Properties of an Arginine Auxotroph of *Salmonella typhimurium*

There are few reports in the literature of arginine auxotrophs of Salmonella. ISEKI and KASHIWAGI[1] obtained an arginine-requiring mutant following treatment of a *Salmonella paratyphi* B strain with nitrogen mustard. STOKES and BAYNE[2] reported a naturally occurring strain of *S. paratyphi* A with a multiple requirement for guanine, cysteine and arginine. GOWEN et al.[3] used two mutants of *S. typhimurium* strain 533 which had multiple requirements that included arginine, and MIYAKE and DEMEREC[4] mentioned an arginine auxotroph in the LT-7 mutator strain of *S. typhimurium*.

More recently LAHR[5,6] has described 39 arg$^-$ mutants of *S. typhimurium*, of which 27 were induced in strain LT-2 by 2-aminopurine.

[1] S. ISEKI and K. KASHIWAGI, Proc. Japan Acad. *33*, 486 (1957).
[2] J. L. STOKES and H. G. BAYNE, J. Bacteriol. *76*, 417 (1958).
[3] J. W. GOWEN, J. STADLER, H. H. PLOUGH, and H. Y. MILLER, Genetics *38*, 531 (1953).
[4] T. MIYAKE and M. DEMEREC, Nature *183*, 1586 (1959).
[5] E. L. LAHR, Carn. Inst. Wash. Yr. Bk. *59*, 438 (1960).
[6] E. L. LAHR, personal communication (1961).

The present report describes an arginine auxotroph isolated in *S. typhimurium* strain LT-22. Auxotroph G-12 was the only mutant which responded to arginine to be isolated in numerous experiments with untreated, ultraviolet-irradiated, and $MnCl_2$-treated cells of *S. typhimurium* strains LT-22 and SW-578. The mutant was isolated in an experiment in which stationary phase cells from an aerated nutrient broth culture of LT-22 were treated with $MnCl_2$[7], grown in broth, screened with penicillin, then plated on mineral medium A[8] supplemented with a limiting concentration of *L*-arginine (1 µg/ml). Arginine auxotroph G-12 was found among the minute colonies which developed alongside the larger, prototrophic, colonies.

Auxotroph G-12 responded only *L*-arginine and showed no growth response to ornithine or citrulline. It was not inhibited by excess *L*-lysine and grew well on complex media. Growth, as measured by colonial diameter on minimal A plates was proportional to *L*-arginine concentration as shown in Figure 1.

Since G-12 was the sole arg$^-$ mutant isolated from penicillin selection experiments it was of interest to see if G-12 itself survived exposure to penicillin screening. Washed cells of broth cultures of G-12 and the LT-22 prototroph were inoculated separately into tubes of liquid minimal medium A containing 50 i.u. penicillin per ml and incubated at 37°. Samples were plated for assays of viable count after 12–24 h of incubation. The results are shown in Figure 2 from which it can be seen that G-12 arg$^-$ cells survived some 50 times better than prototrophic cells. Approximately 56% of the G-12 cells were still viable after 18 h of penicillin screening.

Since mutations to arginine requirement appeared to be rare, tests were made to determine if reverse mutations back to arginine independence (arg$^-$ → arg$^+$) also occur infrequently. In an experiment designed to measure the rate of spontaneous reversion to arginine independence, no reversions were found among 5.45×10^9 cells from 24 independent broth cultures. During the period of growth in broth the G-12 cells had undergone some 28 divisions. Thus, spontaneous reversions to the arg$^+$ conditions must occur at a low rate.

When cells from a broth culture of G-12 were irradiated with ultraviolet light to 11.3% survival, then incubated on minimal A plates supplemented with 1% liquid nutrient broth[9], arg$^+$ reversions arose at the rate of 0.3 per 10^6 survivors. Figure 3 shows UV-induced reversions of G-12 on limitedly enriched minimal A medium. Manganous chloride treatment to 63% survival gave 0.05 arg$^+$ reversions per 10^6 survivors. Nitrous acid treatment gave no reversions over a wide range of survival values.

Using the spot testing method of IYER and SZYBALSKI[10] the effect of chemical mutagens upon G-12 was ascertained. Negative results were obtained with di-epoxybutane, Caffeine, 5-bromouracil + aminopterin, and H_2O_2 + HCHO. β-propiolactone gave a few reversions, and numerous reversions were obtained after treatment with ethyl methane sulphonate as shown in Figure 4.

Transductional tests were carried out using the temperate bacteriophage PLT-22 grown on prototrophic bacteria. G-12 was infected at a multiplicity of 20 and plated on limitedly enriched minimal A. Transductions to arg$^+$ occurred at a rate of 1 per 10^7 phage particles.

Attempted hybridizations of G-12 with other enteric bacteria were performed using mixed cultivation in broth followed by plating on selective media[4], and by cross replication onto selective media[11]. Negative results were obtained in all attempted matings of G-12, and of a spontaneous rough colony variant of G-12, with *Esche-*

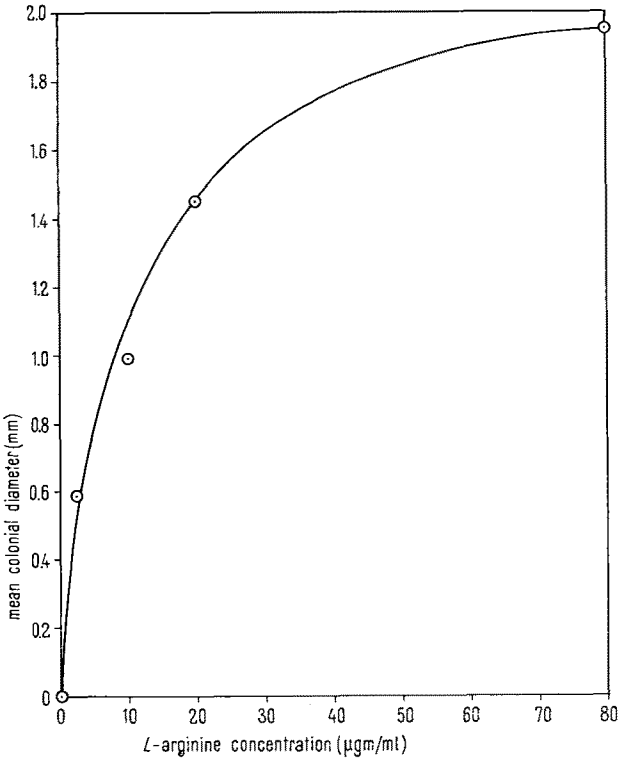

Fig. 1. Growth response of G-12 arg$^-$ on minimal A plates with increasing concentrations of *L*-arginine. Mean colonial diameters are plotted against *L*-arginine concentration. Each point represents the mean of 60–75 measurements made from photographs of the plates.

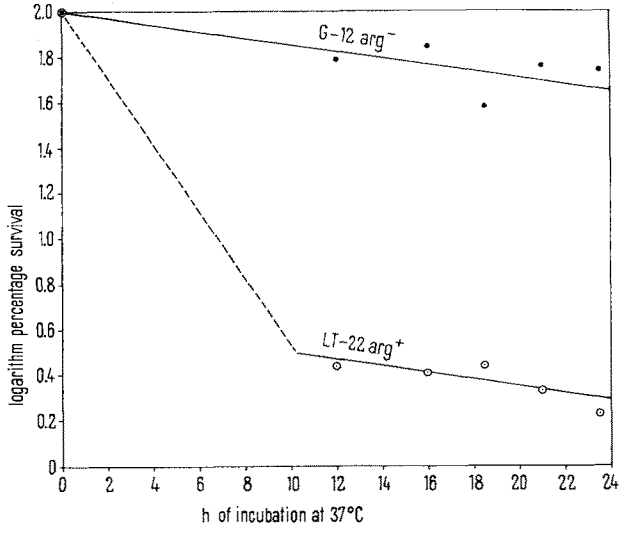

Fig. 2. Differential killing of arg$^-$ and arg$^+$ cells incubated in liquid minimal medium plus 50 i.u. penicillin per ml. Logarithm of percentage survival is plotted against the period of incubation at 37°. Upper line shows survival of the arg$^-$ G-12 cells; lower line that of the prototrophic LT-22 cells.

[7] B. W. HOLLOWAY, J. gen. Microbiol. *13*, 572 (1955).
[8] B. D. DAVIS and E. S. MINGIOLI, J. Bacteriol. *60*, 17 (1950).
[9] E. M. WITKIN, Cold Spring Harb. Symp. Quant. Biol. *21*, 123 (1956).
[10] V. N. IYER and W. SZYBALSKI, J. appl. Microbiol. *6*, 24 (1958).
[11] U. LEUPOLD, Arch. Jul. Klaus-Stiftung, Zürich *30*, 15. Jahresber. S.S.G. 506 (1955).

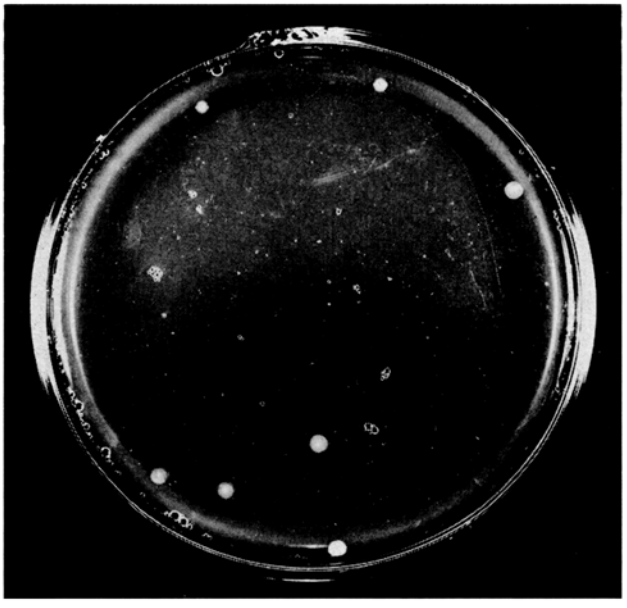

Fig. 3. Arg$^+$ reversions of G-12 induced by UV on minimal A plate with limited broth enrichment.

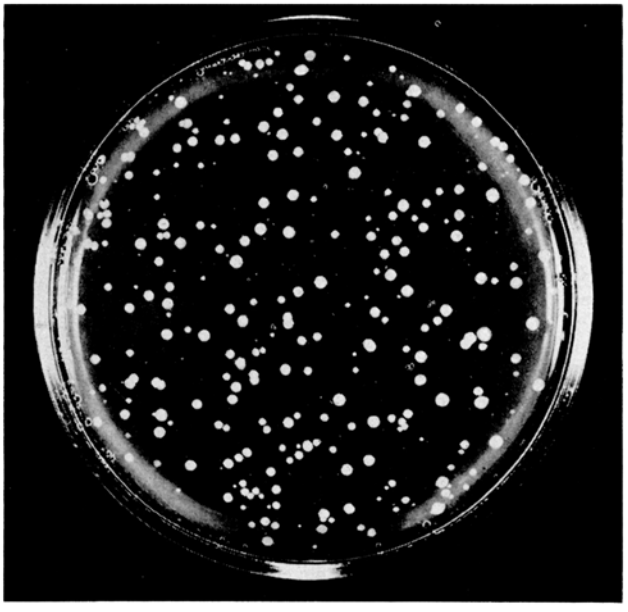

Fig. 4. Reversions of G-12 produced by direct treatment with 0.03 ml of ethyl methane sulphonate on limitedly enriched minimal A medium.

richia coli K-12 strains HfrC and HfrH, with cysteine auxotrophs of *Klebsiella pneumoniae* strains 2 PARK and 8172, and a methionine auxotroph of *K. pneumoniae* strain 418. Strong syntrophism occurred between Salmonella prototrophs and auxotrophs, including G-12, and met$^-$ or cys$^-$ mutants of Escherichia and Klebsiella, probably due to H_2S production by the Salmonellae[12].

Résumé. L'auteur décrit un mutant auxotrophe pour l'arginine chez *Salmonella typhimurium*. Les prototrophes arg$^+$ sont induits par les rayons UV, $MnCl_2$ et méthane sulfonate d'éthyle. Le β-propiolactone est moins efficace pour l'induction des arg$^+$. La transduction avec le bactériophage PLT-22 cultivé sur une souche prototrophe donne arg$^+$ à une fréquence de $1/10^7$ bactériophages. Le mutant arg$^-$ est fécond ni avec les souches d'*Escherichia coli* K-12 HfrC et HfrH ni avec trois souches de *Klebsiella pneumoniae*.

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Institute of Animal Genetics, Edinburgh (England), May 19, 1961.

[12] H. H. Plough, H. Y. Miller, and M. E. Berry, Proc. Nat. Acad. Sci. U.S. *37*, 640 (1951).

[13] I am very grateful to Professor D. G. Catcheside, F.R.S., for his invaluable encouragement and advice, and to the Agricultural Research Council for the award of a Research Studentship.

Uptake of C^{14}-Ethionine by Mouse Foetuses[1]

In connection with our investigations on the incorporation of C^{14}-ethionine into the pancreas, surprisingly high accumulation of radioactivity was observed in the foetuses of pregnant mice.

Various investigators have reported that amino acid analogues have specific effects on the growth of the foetus and are toxic for it[2–4]. It has been suggested that this effect is primarily concerned with protein synthesis of the embryonic cell.

The administration of ethionine—the ethyl analogue of methionine—to male or female rats causes acute pancreatic acinar necrosis[5,6]. The pathogenesis of the pancreatic lesions is apparently related to interference in some phase of methionine metabolism since the changes are prevented by the simultaneous administration of methionine.

Ethionine has been given to pregnant rats in an attempt to induce congenital cystic fibrosis of the young pancreas[4]. This attempt failed but many of the young were born dead, had various congenital malformations, or were undersized. Although the maternal pancreases were severely damaged by the ethionine injections, the foetal pancreas remained essentially normal and the authors therefore questioned the passage of the ethionine across the placenta. Against this background, we should like to report our observations on the uptake and nature of the radioactivity observed in the foetuses after administration of C^{14}-ethionine to pregnant mice.

Two days before expected parturition, pregnant albino mice (body weight 30 g) were injected intravenously with 1 μC C^{14}-*DL*-ethionine (Research Specialties, Richmond, Calif., U.S.A., Spec. act. 1.2 mc/m*M*).

[1] This work was supported by grants from the Swedish Medical Research Council.

[2] C. M. Lee, B. A. Wiseman, S. A. Kaplan, and J. Warkany, J. Arch. Path. *59*, 232 (1955).

[3] C. A. Waddington and M. J. Perry, J. Embryol. exp. Morph. *6*, 366 (1958).

[4] H. Herrman, V. R. Konigsberg, and M. F. Curry, J. exp. Zool. *128*, 359 (1955).

[5] E. Farber and H. Popper, Proc. Soc. exp. Biol. Med. *74*, 838 (1958).

[6] R. C. Goldberg, I. L. Chaikoff, and H. Dodge, Proc. Soc. exp. Biol. Med. *74*, 869 (1950).