Stabilizing Effect of Amino Acids on Bacteriophage T1r+.* (21245)

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Burnet and McKie(1) reported the effect of certain ions on the stability of coli and staphylococcal bacteriophages. Sodium, potassium and ammonium salts increased the susceptibility to inactivation while small amounts of calcium, magnesium or barium salts reversed the influence of the first group. Later Adams(2) studied in detail the stability of the "T" series of coli-dysentery phages with particular emphasis upon phage T5. Essentially his results confirm the findings of Burnet and McKie.

In the course of experiments with T1r+ phage it was found that certain amino acids stabilized this bacteriophage as do calcium and magnesium ions under conditions similar to those of Burnet and McKie(1) and of Adams (2). The rapid inactivation of diluted phage in 0.15 N sodium chloride, distilled water or Davis-Mingioli medium(3) at 37°C was prevented. This finding is reported in the hope that it may be of interest in studies on factors affecting the stability of these agents.

Materials and methods. The T1r+ phage used in these experiments was obtained from Dr. Hotchkiss of the Rockefeller Institute. Stock phage was grown on *E. coli,* strain K-12, in the medium of Davis and Mingioli(3) with and without sodium citrate. At a temperature of 2-4°C phage stocks showed little loss of activity for 5 or 6 months. Phage assays were made on both *E. coli,* strain K-12 and strain B, using the agar layer technic as set forth by Adams(2). The plating medium was a tryptic digest of beef heart agar, 0.5% in sodium chloride. For other details see Table I. All glassware was cleaned with acid dichromate and liberally rinsed with tap and distilled water.

Results. Experiments designed to follow the reproduction of coli phage T1r+ on K-12 employing a small inoculum of phage particles were complicated by the rapid loss of the inoculum at 37°C(4). This inactivation proved to be unrelated to the presence of bacteria and could be demonstrated when phage alone was incubated at 37°C in the medium of Davis and Mingioli, distilled water or .15 N sodium or potassium chloride. The rate of inactivation was essentially the same in each of these diluents.

TABLE I. Effect of Calcium, Mg, and Mn Ions on Inactivation of Phage T1r+ at 37°C.

Diluent	Phage assay* at time 0	1 hr	3 hr
(0.5% glucose)			
† Davis medium (no citrate)	460	40	20
‡ Same + Ca^{++} (.001M)	404	70	28
+ Ca^{++} (.01 M)	490	361	320
+ Mg^{++} (.001M)	440	65	40
+ Mg^{++} (.01 M)	473	325	300
+ Mn^{++} (.001M)	458	73	30
+ Mn^{++} (.01 M)	420	45	35
Broth	465	378	347

* Phage assays are the No. of plaques formed on nutrient agar with *E. coli,* strain K-12 as indicator strain: (*E. coli,* strain B gives essentially the same values).

Each reported figure represents avg of triplicate experimental tubes. Final vol of each tube was 1 ml; of this a 0.1 ml sample was removed for plating after technic of Adams(2) and the phage of remaining 0.9 ml estimated on a second plate by same technic. This partition of sample was followed to insure a countable plate.

† All values expressed as final molar concentration.

‡ Expressed as soluble Ca^{++} which remains after precipitation by phosphate in medium. (Estimated by calculation from solubility constants.) Acid salts were neutralized before addition.

Broth as the suspending medium preserved the infectivity of the phage as did synthetic medium to which calcium or magnesium ions were added at 0.01 M concentration (Table I). Results obtained on addition of calcium, magnesium or manganese to distilled water and to .15 N sodium chloride solution as the suspending fluid were not significantly different from those presented for synthetic medium in Table I.

Certain amino acids, purine and pyrimidine

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TABLE II. Amino Acids as Stabilizers of T1r+ Bacteriophage at 37°C.

Diluent	Phage assay at time 0	1 hr	3 hr
Davis medium (.5% glucose, no citrate)	620	40	7
* + DL-Threonine (.001M)	604	427	330
+ D- Threonine "	585	473	415
+ L- Threonine "	593	484	400
+ DL-Aspartic "	611	436	370
+ D- Aspartic "	603	450	410
+ L- Asparagine "	560	92	12
+ L- Glutamic "	564	500	463
+ DL-Serine "	580	380	345
+ DL-Homoserine "	625	430	370
+ Glycine "	623	53	8
+ DL-Valine "	577	74	32

* Final molar concentration for all compounds. The following compounds exerted no stabilizing effects: results essentially same as seen with glycine and valine (above): DL-Isoleucine, L-Leucine, DL-Methionine, DL-Norvaline, DL-Ethionine, DL-NH_2 Butyric acid, B-OH Butyric acid, L-Lysine, L-Arginine, Adenine, Cytosine, Cytidine, Cytidylic acid, Uridylic acid, Uracil, Guanine, Guanosine.

bases, nucleosides and nucleotides and B-OH butyric acid were tested singly for their effect upon phage stability. It was found that certain amino acids at a concentration of .001 M or .01 M did stabilize the phage while others and compounds of other classes did not (Table II). Glutamic and aspartic acids, serine, threonine and homoserine were effective stabilizers, while asparagine, lysine, arginine and B –OH butyric acid were not. It will be seen that either D- or L-threonine, DL- or D-aspartic acid preserved the infectivity of the phage.

When added together, calcium and aspartate at certain concentration ratios seem to counteract each other. From the data in Table III it will be seen that the concentration of calcium ion sufficient to stabilize phage by itself fails to prevent inactivation in the presence of DL-aspartate, 0.001 M. Aspartate, .0001 M, also counteracts to a slight extent the stabilizing effect of calcium.

TABLE III. Effect of Calcium plus Aspartate on Stability of T1r+ Phage at 37°C.

Diluent	Phage assay at time 0	1 hr	3 hr
Davis medium (.5% glucose, no citrate)	483	36	20
* + Ca^{++} .01M	434	311	297
+ DL-Aspartate .001M	462	277	233
+ Ca^{++} .01M + DL-Aspartate .00001M	450	302	286
+ Ca^{++} .01M + DL-Aspartate .0001M	430	233	182
+ Ca^{++} .01M + DL-Aspartate .001M	446	103	34

* Expressed as soluble Ca^{++} which remains after precipitation by phosphate in medium. (Estimated by calculation from solubility constants.)

Discussion. The effect of the suspending medium upon the stability of bacteriophages of the T series has been studied by several investigators. Burnet and McKie(1) and Adams (2) have reported in some detail the relative instability of these phages in dilute solutions of sodium salts at 37°C and the protection of phage in this unfavorable circumstance by a variety of cations. Adams(2) found that in dilute sodium chloride the rate of inactivation of phage T5 was greatly decreased by the presence of .001 M concentrations of Ca, Mg, Ba, Sr, Mn, Co, Ni, Zn, Cd or Cu.

The instability of phage T1 has been mentioned by Puck(4) as a property apart from the reversible inactivation under particular conditions with which he dealt at length. The reversible inactivation was shown to take place on contact with bacteria after a period of preincubation of phage alone in buffer. The inactivation dealt with in the present study is not reversible by addition of cations or amino acids after preincubation of the phage and is unrelated to the presence of bacteria. It has been demonstrated to take place at 37°C in a synthetic medium with or without glucose or citrate, in distilled water or in .15 N sodium or potassium chloride. Although addition of calcium or magnesium in .01 M concentration results in increased stability of the phage, this effect could not be produced with manganese chloride or sulfate. No other metals were tried.

As effective as cations in making the phage more stable were the amino acids, threonine, serine, homoserine, aspartic acid and glutamic acid. These are all amino acids having two potentially ionized acidic groups at pH 7.0. Neither neutral nor basic amino acids could be demonstrated to increase the stability of phage. Indeed asparagine, in which one of the carboxyl groups of aspartic acid has been substituted with an amino group, is not effective. From this it might be suggested that two

acidic groups or the combinations –OH and –COOH on the amino acid are essential in stabilizing the bacteriophage.

The separate effects of calcium and aspartate are suppressed by combination of the two at certain levels. The binding of metals by amino acids is a well established fact and appears a reasonable explanation for the mutual suppression.

Summary. 1. T1r+ phage is rapidly and irreversibly inactivated at 37°C in distilled water, .15 N sodium or potassium chloride or in Davis medium. 2. Addition of calcium, magnesium or the amino acids serine, threonine, homoserine, aspartate or glutamate results in stabilization of phage in the diluents mentioned above.

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Limits of Absorption of Orally Administered Vitamin B_{12}: Effect of Intrinsic Factor Sources.* (21246)

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Studies of patients having pernicious anemia(2,3) and of persons with total gastrectomy(4,5) have shown that when vit. B_{12} is given orally in doses of 0.5 μg its absorption from the gastrointestinal tract is dependent upon the presence of a gastric substance, presumably the Castle-defined intrinsic factor(6). However, the role of intrinsic factor in facilitating absorption of vit. B_{12} has not as yet been clarified and, moreover, the pattern of absorption of this vitamin in subjects with normal gastric secretion has not been defined.

In the present investigation, a study has been made of the absorption of vit. B_{12} in individuals with clinically normal gastric function. Graded doses of Co^{60}-labeled vit. B_{12}† were administered orally and the total amount of radioactivity excreted in stool samples measured by methods previously reported(2,5).

Results. 0.5, 2, 5, and 10 μg amounts of labeled B_{12} were given in a series of doses at various time intervals to subjects who were either fasting or between meals. Each dose contained 0.110 μc of Co^{60}. No test was repeated with a given subject using the same dose, although most subjects received more than one dose level. It was found (Table I) that as the amount of administered vit. B_{12} was increased, the per cent of Co^{60} excreted in the stools increased. The amount of vit. B_{12} presumed to be absorbed, a figure obtained by subtracting the apparent B_{12} excreted from the total amount administered (Table I), was no greater with 10 μg than with a 5 μg dose, an average value of approximately 1.6 μg being the calculated absorption at both dose levels.

TABLE I. Fecal Excretion of Co^{60} following Graded Doses of Co^{60} Vit. B_{12} Given to Normal Subjects.

Dose in μg	No. of tests	% Co^{60} excreted Range	Avg	μg vit. B_{12} absorbed (calculated) Range	Avg
.5	15	57-19	35	.22- .41	.34
2.0	12	69-30	48	.61-1.44	1.03
5.0	15	87-50	67	.73-2.50	1.65
10.0	5	93-70	84	.68-2.97	1.63

The effect of a test meal on vit. B_{12} absorp-

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