TECHNICAL PAPERS

The Amino Acid Content of Bacteriophage

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Methods of partition chromatography (*1*) require such small quantities of material that they provide a way to investigate the amino acid content of purified viruses which are available only in minute amounts. One of the most convenient virus systems to be studied in this way consists of bacteriophages and their host organisms.

TABLE 1

AMINO ACID CONTENTS OF *E. coli* AND T (4) BACTERIOPHAGE

Amino acid	Percentage*	
	In *E. coli*	In T(4)
Aspartic acid	9.57	11.97
Glutamic acid	9.59	11.97
Serine	4.88	4.77
Glycine	7.94	7.34
Threonine	5.31	7.00
Alanine	8.40	9.40
Valine	5.00	6.51
Methionine	2.92	< 1.3
Phenyl alanine	4.80	4.16
Isoleucine	4.63	3.90
Leucine	8.68	6.51
Tryptophane	1.29	....
Proline	3.02	5.00
Tyrosine	4.33	3.74
Arginine	8.21	6.51
Lysine	8.26	8.46
Histidine	3.26	< 2.6

* Expressed as percentage of total amino acids found on paper.

With this in mind, we have been analyzing *E. coli* and several of its bacteriophages. Results of chromatographic analysis of the organisms are being published elsewhere (*2*). Preliminary results of a corresponding analysis of the T(4) strain of its bacteriophage are reported here.

The quantitative methods of filter paper chromatography employed (*2, 3*) have been the same as those described in connection with the analysis of *E. coli*. The preparation of bacteriophage was one that had been purified by ultracentrifugation. It was hydrolyzed in the same way as the bacteria and otherwise handled in the same fashion; the amino acid content determined for it is listed in Table 1, along with the results on *E. coli* calculated in terms of the per cents of each amino acid referred to the total amount of amino acid found on the chromatogram. Evidently the same amino acids are present in both the bacterial and the bacteriophage suspensions. There are some differences in the relative amounts of these amino acids in the two preparations, but, for the reasons stated below, these differences cannot yet be considered as significant.

The greatest uncertainty in this work lies not in the results of the analytical procedures but rather in the difficulties in getting sufficiently pure bacteriophage suspensions. Electron micrographic observation indicated that the suspension of bacteriophage consisted predominately of the well-known sperm-like particles of this virus; but, though this demonstrates that the preparation had been largely purified by the ultracentrifugation to which it had been subjected, it is not an adequate proof of chemical purity. The final proof of purity must be consistency of analytical results with repeated purification. Considerable progress is still being made in the development of methods that can yield relatively large amounts of increasingly pure coli bacteriophages. Analytical work is being carried out on several phages purified by these methods. Its results will subsequently be described in detail.

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The Combined Effect of Potassium Iodide and Streptomycin on Established Tuberculosis in Guinea Pigs

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It is a well-known clinical observation that iodides cause tubercle bacilli previously absent to appear in the sputum of patients with pulmonary tuberculosis. Jobling and Petersen (*2*) reported in 1914 that iodine will combine with the unsaturated fatty acids obtained from tubercle bacilli and will neutralize their ferment-inhibiting properties. Subsequent to this neutralization, ferment action ensues within areas of caseation with liberation of the bacilli and their appearance in the sputum. They also stated that iodine might serve another purpose by facilitating solution and absorption of the caseous matter, thus exposing the bacilli, which otherwise might be inaccessible to the influence of an effective therapeutic agent. In the light of this very significant work and in view of the

relatively poor results obtained in the treatment of fibrocaseous tuberculosis with streptomycin alone (*1*), it seemed highly desirable to test the combined effect of potassium iodide and streptomycin against established tuberculosis in guinea pigs.

First of all, it appeared essential to determine *in vitro* any possible effect of KI on the antibiotic potency of streptomycin. This was done by mixing varying concentrations of streptomycin with serial dilutions of potassium iodide ranging from .01 to 1.6 M concentration in infusion broth at 56° and 95° C for 7 min. The antibiotic titer of these mixtures was tested by using a fast-growing strain of tubercle bacillus. No alteration in the potency of the streptomycin was observed when compared with controls after incubation.

In view of these results, four groups of young guinea pigs, varying from 350 to 450 gm in weight, were inoculated in the groin with 1 cc of an aqueous suspension of tubercle bacilli (H37RV) containing 0.30 mg/cc. Seven pigs served as a control group; the other three groups (10 pigs each) were treated, respectively, with potassium iodide alone, with streptomycin alone, and with both streptomycin and potassium iodide. Treatment was begun 21 days after inoculation. The dosage of potassium iodide was calculated on the basis of 80 mg/kg of body weight/day and was given in a weak aqueous solution (16 mg/cc) by stomach tube once daily. The dosage of streptomycin was calculated on the basis of 12,500 μg/kg of body weight/day and was injected intramuscularly at 6-hr intervals. During the course of four weeks treatment, the inguinal nodes in the streptomycin-potassium iodide group remained significantly smaller than those in the other three groups. At the end of four weeks of treatment and the seventh week of infection, all animals were sacrificed and autopsied. On gross examination, the controls and KI guinea pigs showed heavy tuberculous infection of all the viscera, in the streptomycin group five pigs showed spread to the organs, whereas in the streptomycin-KI group the organs were entirely free from infection.

A subsequent survival experiment was run, using young pigs ranging from 350 to 450 gm in weight. This time three groups of guinea pigs were used: 15 in a control group, 15 in a group treated with streptomycin alone, and 16 in a group treated with both streptomycin and potassium iodide. Inoculation with H37RV was carried out in exactly the same manner as in the preceding experiment. Treatment was delayed, however, until the end of the fourth week of infection. The potassium iodide dosage was the same as that used in the first experiment. The streptomycin was increased to three times the former dosage. Treatment was carried on for a period of five weeks and then discontinued. At the end of the twelfth week of infection, 13 of the 15 in the control group were dead, 5 of the 15 in the streptomycin group were dead, and only 1 of the 16 in the streptomycin-KI group had succumbed. At this time two animals in each group were sacrificed and autopsied for the purpose of obtaining microscopic sections from the three groups simultaneously. Results of microscopic studies will be reported in a future communication. Excluding the two pigs sacrificed from each group, the deaths from tuberculosis at the end of the 15th week of infection were: controls, 13 of 13 animals; the streptomycin group, 6 of 13; the streptomycin-KI group, 2 of 14. The respective mortality percentage rates were thus 100%, 46.1%, and 14.3%.

These results offer many interesting possibilities for further investigation. Clinical tests are now in progress.

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Graphite Bearings for Mechanical Stirrers

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Machined graphite bearings placed at strategic points in the mercury seal of a laboratory stirrer permit extremely rapid stirring with a minimum of attention. Graphite can be turned down on an ordinary lathe that is equipped with a chuck or collets. Precision tooling is not necessary. These bearings are machined in such a manner as to allow free movement of the bearing surfaces without permitting the stirrer shaft to wobble. Because of the variations in glass tubing sizes, it is found advantageous to tailor each set of bearings to fit the seal for which they are intended.

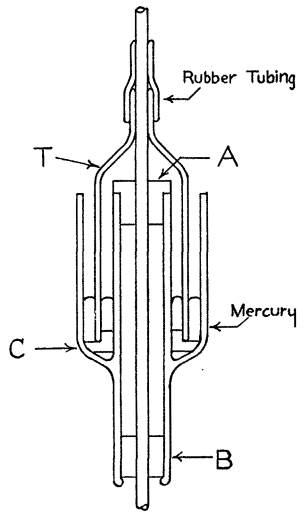

FIG. 1

The cross section of such an assembly (Fig. 1) shows a set of three bearings, which has been found to be the most stable arrangement. Bearings A and B furnish dual bearing surfaces between the stirrer shaft and the inner tube of the mercury seal. Bearing C braces the lower end of tube T, which rotates with the stirrer shaft. The lower end of the inner tube must be slightly constricted by fire polishing to prevent bearing B from fall-