JCI The Journal of Clinical Investigation

PURITY STUDIES ON POLYPEPTIDE ANTIBIOTICS: BACITRACIN

Lyman C. Craig, … , J. Delafield Gregory, Guy T. Barry

J Clin Invest. 1949;28(5):1014-1017. https://doi.org/10.1172/JCI102131.

Research Article

PURITY STUDIES ON POLYPEPTIDE ANTIBIOTICS: BACITRACIN [1]

By LYMAN C. CRAIG, J. DELAFIELD GREGORY, AND GUY T. BARRY

(From the Laboratories of The Rockefeller Institute for Medical Research, New York, N. Y.)

As part of a broader study on Counter-current Distribution, the question of the purity of available polypeptide preparations has been taken up. We have found it possible to fractionate gramicidin, tyrocidine, gramicidin-S, and bacitracin samples into several different polypeptide components. In each case, except with the tyrocidine fractions which have not been studied antibiotically as yet, a variation in activity with the different components has been noted. They differ also in the amino acids which they contain as well as in the proportion of these amino acids.

Bacitracin for the most part has been furnished by the Commercial Solvents Co. We are indebted to them and to Miss Johnson and Dr. Meleney for the bioassays. Bacitracin has not proved to be the most ideal type of substance to distribute because of its tendency to give skewed curves and because of its lack of stability. In attempts to distribute it in neutral 2-butanol/water, the curves obtained were typical transformation curves. 2-Butanol/water acidified with acetic acid was much more satisfactory and showed only slight transformation during a run. A typical result is shown in Figure 1. This was made with a sample of 46 unit material. A calculated curve superimposed on the weight curve showed the latter to be somewhat skewed. An activity curve also was skewed but seemed to indicate a single active substance being present in the major component. A considerable band of low activity occurred to the right. Absorption spectrum measurement indicated mixtures in tubes 40 to 60.

An interesting feature of this distribution is that a maximum activity of 66 units was indicated. This was on a dry-weight basis when dried at 100° in vacuum. Material recovered from the main band by freeze-drying was a white, highly hygroscopic powder which had an activity slightly less than the maximum obtained from the curve. That it had lost a certain amount of activity through denaturation on isolation was confirmed by redistribution. In this case a small percentage of material of low activity appeared on the left of the main band.

Hydrolysis in 6 N hydrochloric acid followed by paper chromatography gave spots corresponding to phenylalanine, leucine, isoleucine, cystine, valine, histidine, ornithine, lysine, and glutamic and aspartic acids. Strong evidence is thus furnished that the active principle is a polypeptide of considerable size.

At about this stage of our work certain toxic manifestations were encountered by others on the clinical side and it became desirable to have more careful chemical investigations in order to see if some closely related toxic substance could be removed by fractionation. This problem can be approached in two ways, namely, higher numbers of transfers or a change to a more specific system. We have investigated both approaches.

An all-glass apparatus of simple construction has been devised which is shown in Figure 2. This particular one contains 108 units but it would appear that this number could be extended several

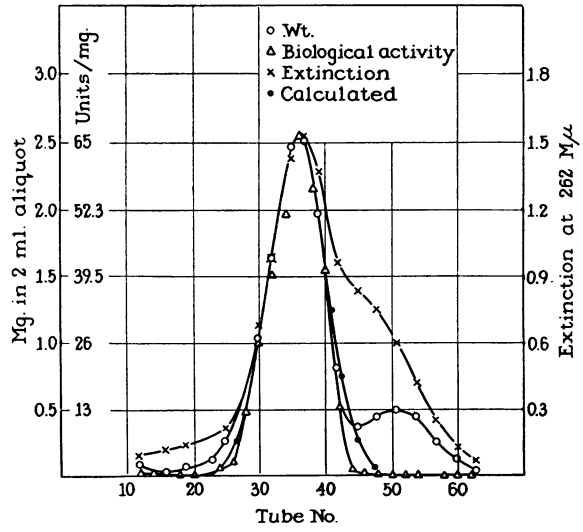

FIG. 1. 85 TRANSFER DISTRIBUTION OF BACITRACIN IN 2-BUTANOL/3% ACETIC ACID

[1] Presented at the Second National Symposium on Recent Advances in Antibiotics Research held in Washington, D. C., April 11–12, 1949, under the auspices of the Antibiotics Study Section, National Institutes of Health, Public Health Service, Federal Security Agency.

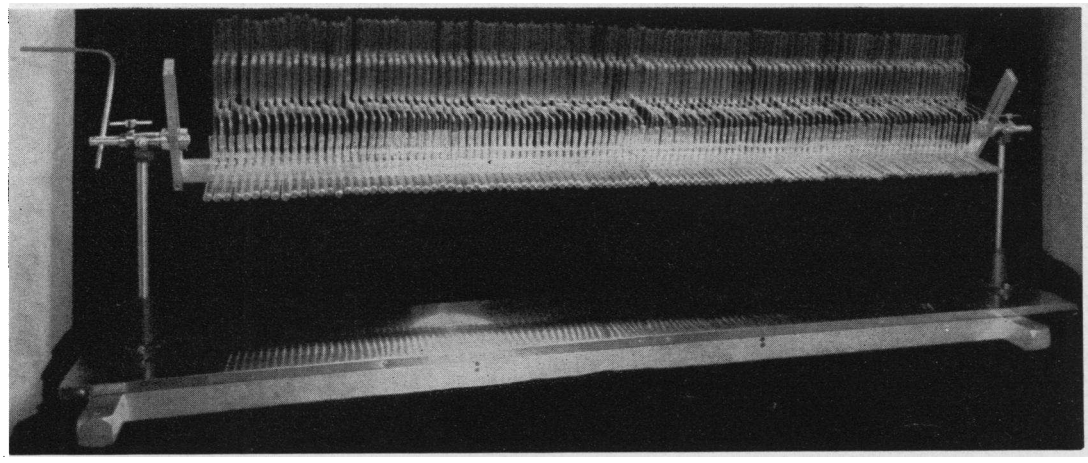

FIG. 2. GLASS COUNTER-CURRENT DISTRIBUTION APPARATUS CONTAINING 108 TUBES

fold if necessary for a specific problem. Enlarging is merely a matter of adding individual units. An interesting feature is that strong acids such as hydrochloric acid can be used.

A run on bacitracin using a system made with methanol, 0.1 N hydrochloric acid and chloroform gave the pattern shown in Figure 3. The overall aspects are not too different from those with the first system. Inactive material is shown at fractions 140 to 120 and a certain amount of foreign material is demonstrated on the left. Somewhat more inactivation was encountered during the run than with the acetic acid system. When inactivation in acid solution occurs, the transformation products appear on the left. When inactivation in neutral or alkaline solution occurs, the products appear on the right. A hydrochloride with rather nice properties was recovered from the peak tubes.

At least one sample of bacitracin was not entirely stable in the solid state. This sample origi-

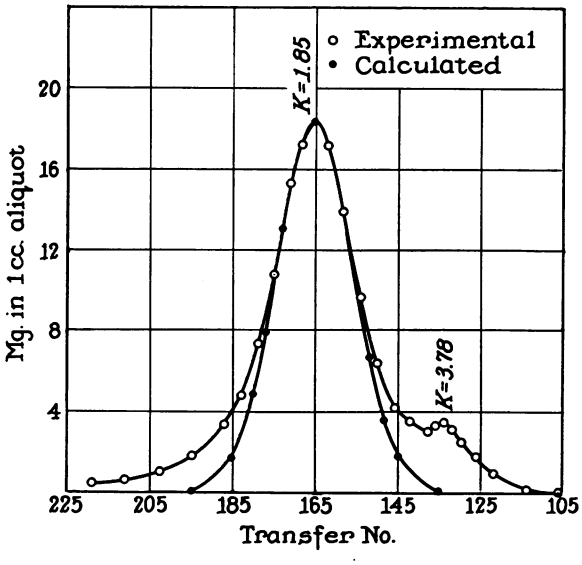

FIG. 3. DISTRIBUTION OF BACITRACIN IN A SYSTEM CONTAINING CHLOROFORM, METHANOL AND 0.1 N HYDROCHLORIC ACID

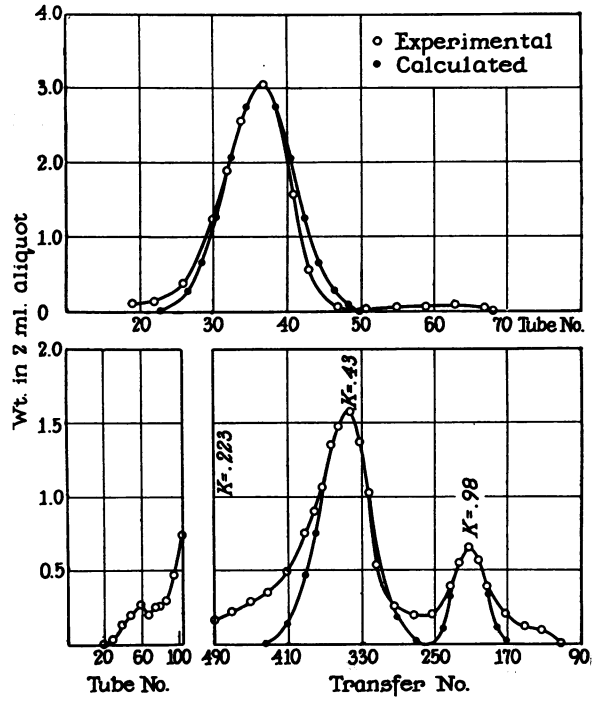

FIG. 4. COMPARATIVE DISTRIBUTIONS OF BACITRACIN IN 2-BUTANOL/3% ACETIC ACID

nally had an activity of 55 units and gave quite a good pattern, Figure 4, upper pattern, with 75 transfers and the acetic acid system. Only a small amount of inactive material on the right and left was obtained. After standing in the cold room for about nine months, the lower pattern was obtained with the same system but with the application of 490 transfers. The activity of the sample after nine months was 47 units. The fractions were recovered as usual by freeze-drying and, to our surprise and gratification, material recovered from the main band on one run had definite organization as shown in Figure 5. None of the fractions recovered from other regions showed this organization. Furthermore, the recovered material had an activity of 60 units without further drying.

It would now appear fairly certain that this material is either a single substance or a mixture of very closely related substances. The evidence is not as rigorous as we would like because of inherent instability but at least the experience in two different systems points toward something definite on which to base future work.

Hydrolysis in boiling 6 N hydrochloric acid for 24 hours, evaporation of the excess hydrochloric acid, and distribution of the mixed hydrochlorides

FIG. 5. APPEARANCE OF PURIFIED BACITRACIN

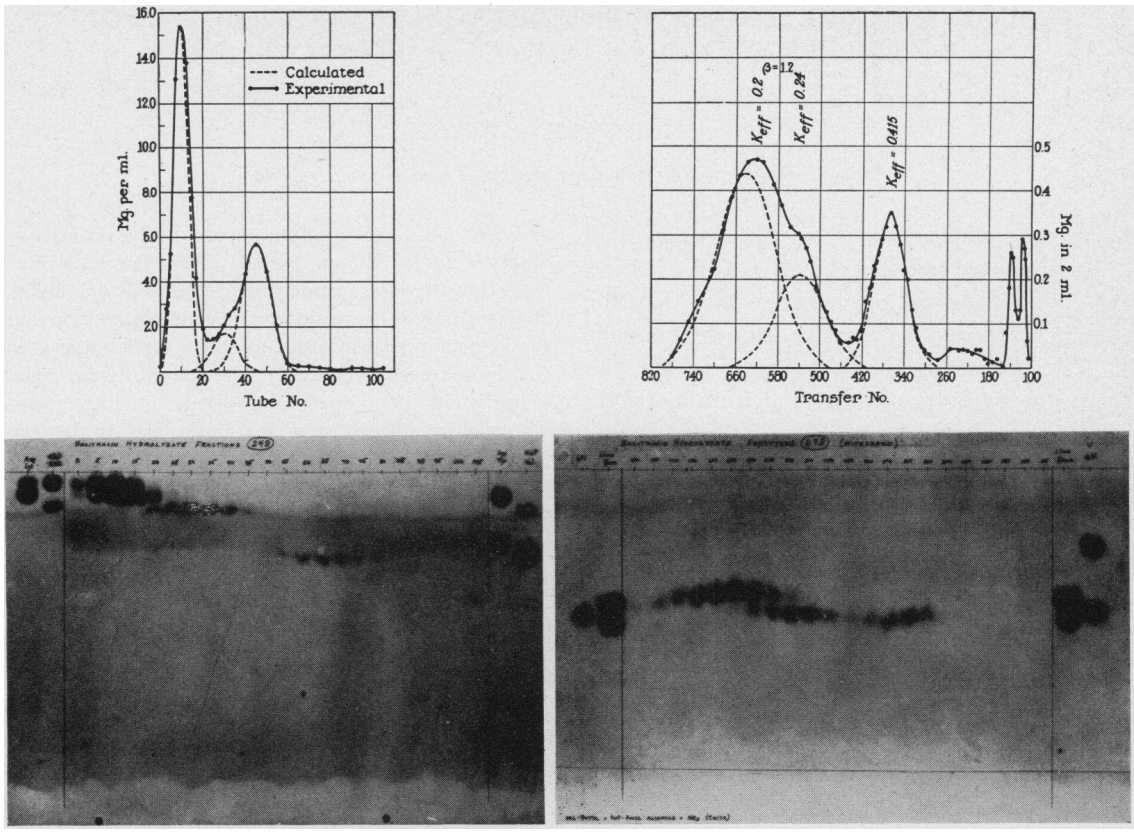

FIG. 6. DISTRIBUTION OF THE HYDROLYSIS PRODUCTS OF BACITRACIN

in a 2-butanol/ammonium acetate system has given the pattern shown in Figure 6. The bands from right to left are a dipeptide containing phenylalanine and isoleucine; peptide material containing phenylalanine, isoleucine, and apparently ornithine; phenylalanine; leucine; isoleucine; ammonium chloride; histidine; and, finally, a mixed band containing cystine, lysine, and aspartic and glutamic acids. Redistribution in other systems has permitted further resolution of the mixed band. Spotting of every tenth fraction on a broad paper chromatogram permits ready control of the separation.

The original "valine" spot has been isolated in crystalline form from tubes 60–70 but it is different from valine. It appears to be absent from the most highly purified material. All the spots indicated by paper chromatography have been isolated in crystalline form with the correct carbon and hydrogen analysis except lysine and ornithine. In addition, peptides have been isolated. The amino acids isolated were of the *l*-, *d,l*- and *d*-configurations, as follows: *l*-histidine, partially racemic *l*-leucine, *l*-cystine and *l*-glutamic acid, *d,l*-phenylalanine, *d,l*-aspartic acid, and partially racemic *d*-isoleucine.