THE EXAMINATION OF A BACTERIOLOGICAL PEPTONE

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Peptone, by reason of its amino-acid content and other nitrogenous compounds, is an important ingredient of microbiological culture media. A knowledge of the constituent amino-acids and peptides is desirable and is a necessary preliminary to obtaining uniformity in bacteriological media.

Paper chromatography has been used previously for the analysis of both the partial and complete hydrolysates of proteins and solvents such as phenol, collidine[1], butanol-acetic acid-water[2] and butanol-formic acid-water[3] systems have been used. McFarren[4] found that buffering the paper as well as the solvent gave reproducible results. Levy and Chung[5] reported a successful two-dimensional chromatographic separation on Whatman No. 1 paper using butanol-acetic acid-water system as the first solvent, followed by spraying the dried paper with a buffer of pH 8·3 and developing in the second direction with *meta*-cresol-phenol (2 : 1) saturated with a buffer of pH 8·3.

Experimental

Analar chemicals and solvents were used throughout.

Chromatograms were formed by the ascending technique on Whatman No. 1 paper ($11\frac{1}{4}$ in. × $18\frac{1}{2}$ in.) in jars covered by glass plates. A seal was effected with Apiezon grease. The atmosphere within the jar was saturated with the solvent and water vapours by lining the jar with filter paper wetted with water saturated with the solvent[4,6]. When equilibrium was established, the solvent saturated with water or buffer solution was introduced into the dish, in which the chromatogram paper stood, down a thistle funnel passing through the lid and extending into the dish itself. The jars were lagged to reduce temperature variations.

Method

When a known mixture of twenty-two amino-acids was submitted to the separation of Levy and Chung[5] the spots showed "heading". Better results were obtained when a butanol-acetic acid-water mixture[2] was used as the first solvent. The paper was dried at 60° C. for 30 minutes[7], sprayed with a buffer of pH 6·2[4], and again dried. A two-dimensional separation was effected by using phenol saturated with a buffer of pH 6·2 as the second solvent and the positions of the amino-acids were revealed by dipping the dried chromatograms in a 0·25 per cent. solution of ninhydrin in acetone. The paper was finally dried at 60° C. for 15 minutes.

This system gave compact spots and separated methionine from leucine and *iso*leucine, but the separation of these latter amino-acids was

not complete, as shown in Figure 1. When the mixture of amino-acids was oxidised by 30 per cent. hydrogen peroxide[8], cysteic acid occupied a position below ornithine and methionine sulphone, slightly below and overlapping hydroxyproline.

Examination of a Bacteriological Peptone

Three batches A, B and C, of "Oxoid" Bacteriological Peptone (Code No. L 37) were examined qualitatively using the technique described above. Peptone gave a complex chromatogram and therefore the method of Fromageot *et al.*[9] was adopted to separate 1 ml. of a 17 per cent. w/v peptone solution into four fractions corresponding roughly to the acidic, basic, aromatic and neutral amino-acids and peptides. Each fraction was evaporated to dryness below 40° C. under vacuum and the residue dissolved in 1 ml. 10 per cent. *iso*propanol[10]. 0·003 ml. of each solution was applied to a paper buffered at pH 6·2 and developed with phenol saturated with the buffer. Five applications of the solution of the aromatic fraction of batch C were found to be necessary to obtain a sufficiently intense colour reaction. The original peptone and known amino-acid were included for comparison as shown in Figure 2. The three batches gave the same general picture except that batch C showed a more intense basic fraction and a weaker aromatic fraction. Batch B also showed a weak aromatic fraction. When these fractions were subjected to two-dimensional chromatography the pattern of the spots obtained for the basic and neutral fractions of the three batches showed a similar distribution, as illustrated in Figures 3 and 4, differing slightly in the diffuseness of the peptide spots. Free lysine and arginine and a spot corresponding to the position of ornithine were found in each basic fraction. In the neutral fractions serine, glycine, threonine, alanine, valine, methionine, proline, α-aminobutyric acid and leucine and *iso*-leucine were present. The acidic fractions were similar for each batch of peptone and showed a particularly large diffuse area, as in Figure 5. Tyrosine and phenylalanine were present in each of the aromatic fractions but the fraction from batch B did not show the four diffuse areas, numbered 2, 3, 4 and 5 in Figure 6, which were present in the batches A and C.

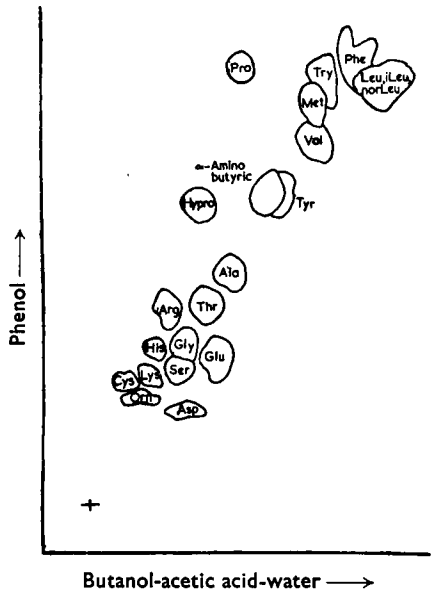

FIG. 1. Two dimensional chromatogram of a known mixture of amino-acids.

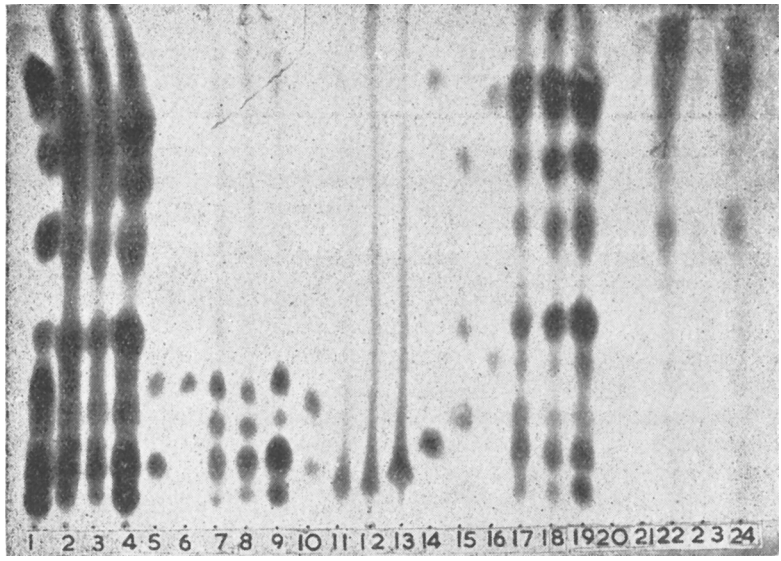

FIG. 2. One dimension chromatogram of peptone and its fractions on buffered paper (pH 6·2).

1. The mixture of amino-acids.
2. Peptone. (Batch A).
3. Peptone. (Batch B).
4. Peptone. (Batch C).
5. Lysine and Arginine.
6. Histidine.
7. Basic fraction. (Batch A).
8. Basic fraction. (Batch B).
9. Basic fraction. (Batch C).
10. Aspartic and glutamic acids.
11. Acidic fraction. (Batch A).
12. Acidic fraction. (Batch B).
13. Acidic fraction. (Batch C).
14. Serine and leucine.
15. Glycine, alanine and valine.
16. Threonine and methionine.
17. Neutral fraction. (Batch A).
18. Neutral fraction. (Batch B).
19. Neutral fraction. (Batch C).
20. Tyrosine and phenylalanine.
21. Tryptophan.
22. Aromatic fraction. (Batch A).
23. Aromatic fraction. (Batch B).
24. Aromatic fraction. (Batch C).

Hydrolysates of the Fractions

Each fraction was hydrolysed by mixing equal volumes of the *iso*-propanol solution with concentrated hydrochloric acid and heating at 100° C. for 15 hours[11]. The hydrochloric acid was removed under vacuum and the dry residue dissolved in 10 per cent. *iso*propanol. Each hydrolysate, and the hydrolysate oxidised with hydrogen peroxide, was submitted to the two-dimensional chromatography described.

The following amino-acids were found in each of the fractions from the three batches of peptone: lysine, arginine, aspartic acid, glutamic acid, serine, glycine, threonine, alanine, hydroxyproline, proline, valine, methionine, leucine, *iso*leucine, phenylalanine and tyrosine. The basic fractions yielded two spots corresponding to ornithine and histidine and the spot due to tyrosine was very faint. Each neutral fraction showed the presence of α-aminobutyric acid and an unidentified spot having an R_F similar to tyrosine in phenol, and similar to alanine in the butanol-acetic acid-water system. Batch B yielded a second unidentified spot with

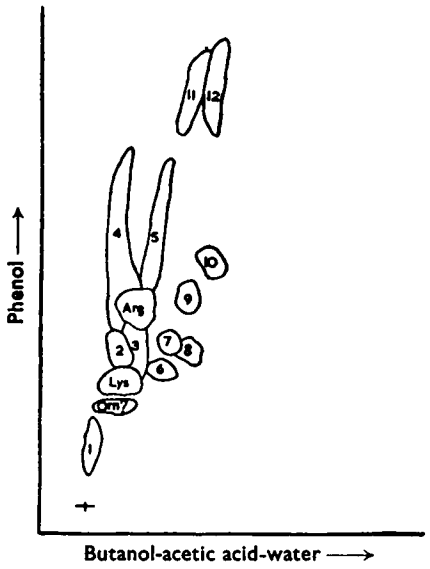

FIG. 3. Two dimensional chromatogram of the basic fraction. (Batch A).

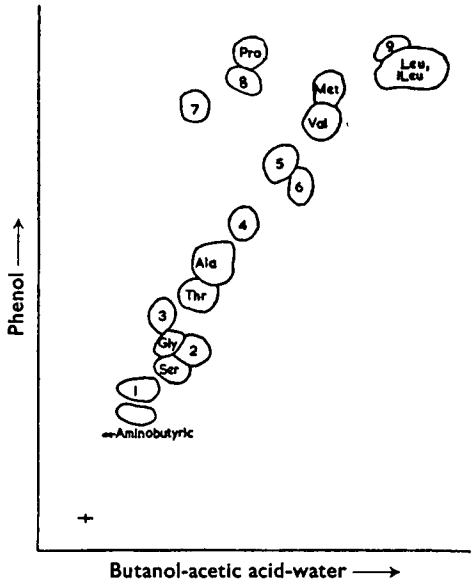

FIG. 4. Two dimensional chromatogram of the neutral fraction. (Batch A).

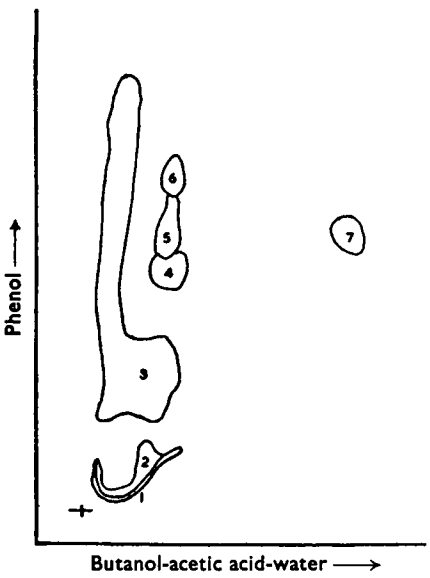

FIG. 5. Two dimensional chromatogram of the acidic fraction. (Batch A).

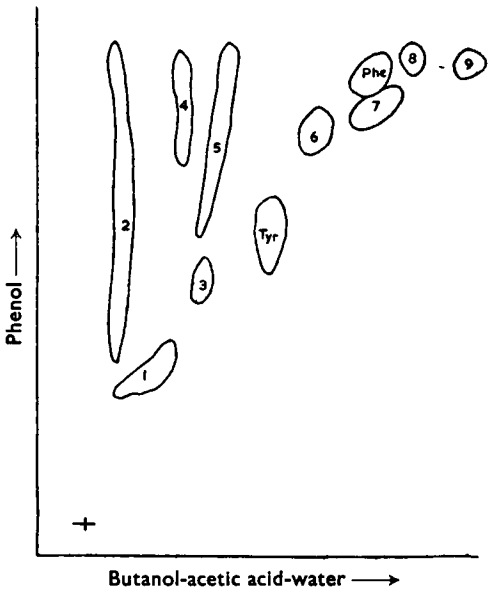

FIG. 6. Two dimensional chromatogram of the aromatic fraction. (Batch A).

an R_F value in phenol similar to valine, and slightly below α-aminobutyric acid in the butanol-acetic acid water system.

In the hydrolysate of the aromatic fraction a faint spot corresponding to ornithine was found but the spots due to tyrosine and phenylalanine were intense. The hydrolysed basic fraction of the peptone gave a more intense reaction for the basic than for the acidic amino-acids, the reverse being true for the acidic peptone fraction.

Tryptophan was found to be present in each batch of peptone when the hydrolysis was effected with barium hydroxide[5].

Discussion

The separation of the peptone into fractions by the method of Fromageot *et al.* will depend largely on the predominating amino-acids which will govern the character of the peptide, and so it is possible, for example, to obtain basic peptides containing acidic amino-acids. The adsorption on charcoal of peptides containing aromatic residues was not found to be complete; a similar result was obtained by Sanger and Tuppy[12]. Very little difference in the amino-acid content was found between the three batches of peptone which were examined qualitatively although there appears to be some variation in the peptide content. The chromatographic patterns obtained by one and two-dimensional chromatography may afford a convenient method of comparing and standardising peptones for the ninhydrin-positive substances. The variation in the spots, corresponding to peptides, may be due to variations in the manufacturing procedure.

Summary

1. The use of butanol-acetic acid-water system as the first solvent, spraying the dried paper with a buffer of pH 6·2 and developing in the second direction with phenol saturated with buffer pH 6·2 gave a well defined separation of a known mixture of twenty-two amino-acids.

2. One and two-dimensional chromatograms of the separated basic, acidic, neutral and aromatic fractions of the peptones gave a number of free amino-acids, but variations occurred in the ninhydrin-positive spots due to peptides of the three batches of bacteriological peptone examined.

3. The hydrolysed peptone yielded 20 amino-acids and one unidentified spot in all the three batches and a second spot in Batch B.

References

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