STUDIES ON THE AMINO-ACID METABOLISM OF *MYCOBACTERIUM TUBERCULOSIS*

I. PRELIMINARY INVESTIGATION BY PAPER CHROMATOGRAPHY*

by

G. PAULETTA AND A. DEFRANCESCHI

Laboratorio di Microbiologia, Istituto Carlo Erba per ricerche terapeutiche, Milan (Italy)

Recent studies revealed that the amino-acid metabolism of bacteria is closely related to the mechanism of antibacterial action[1]. A new convenient method to follow such metabolism is offered by paper partition chromatography[2,3,4,5]. With the aid of this method we succeeded in following the amino-acid composition of a number of *Mycobacterium* cultures, at different periods of growth and in different fractions; namely, in the culture filtrate, in the cell homogenate, and in the cell hydrolysate.

MATERIALS AND METHODS

Organisms and growth media

The following strains of *Mycobacterium* were used:

1. *Mycobacterium tuberculosis* var. *hominis* H37Rv (Streptomycin sensitive).
2. *Mycobacterium tuberculosis* var. *hominis* H37Rv (Streptomycin resistant).
3. *Mycobacterium tuberculosis* var. *hominis* H37Ra (Attenuated variant).
4. *Mycobacterium tuberculosis* var. *hominis* Tb1
5. *Mycobacterium tuberculosis* var. *bovis* P.
6. *Mycobacterium avium* Panisset.

The medium employed in the majority of experiments was the PROSKAUER AND BECK synthetic medium, modified by YOUMANS[6]. In some tests this medium was modified by substitution of asparagine (0.5%) with NH_4Cl (0.405%), so that the nitrogen percentage in both media remained the same. 250 ml Erlenmeyer flasks containing 100 ml of medium were employed. Each flask was inoculated with a loopful of a 14-day pellicle surface growth, and incubated at 37.5° C. A number of flasks sufficient to provide 1 g of wet cells was collected at various days of growth.

Preparation of culture fractions

Each culture was divided in three fractions for the chromatographic analysis:

1. culture filtrate
2. cell homogenate
3. cell hydrolysate

The scheme followed for the preparation of the various fractions is illustrated in Table I. The POTTER-ELVEHJEM[7] glass homogenizer was used for cell disruption at the beginning of this work. Later, since the same result was obtained by heating the cell suspension at 100° C for 10 minutes, as pointed out already by GALE[8], it was decided to adopt this second easier method. Cell hydrolysis was made according to the procedure described by BLOCK[9].

* Part of the material in this paper has been presented before the XII International Congress of Pure and Applied Chemistry—New York City—September 10, 1951.

References p. 282.

TABLE I

SCHEME FOLLOWED FOR THE PREPARATION OF THE THREE FRACTIONS FROM *Mycobacterium* CULTURES

The culture was filtered through sintered glass (Jena 3G4)

filtrate	cells
Filtered again through Pyrex U.F. sintered glass filter. 10 ml of the filtrate were evaporated to dryness *in vacuo* at 40–50° C. The dry residue was dissolved in 1 ml of 10% *iso*propanol.	washed free from the medium 5–6 times with 10 ml portions of distilled water dried on the filter and then divided in two parts.
Culture filtrate I	
	A portion of about 500 mg was placed into a weighed centrifuge tube, and then weighed wet. A volume of water corresponding to ten times the weight of the cells was added and the resulting suspension was heated in boiling water for ten minutes, then centrifuged and filtered through Pyrex U.F. filter.
	Cell homogenate II
	500 mg of wet cells were hydrolysed with 25 ml of *N* Hydrochloric acid. The residue obtained after hydrolysis was taken up with a volume of 10% *iso*propanol, corresponding to ten times the weight of the cells.
	Cell Hydrolysate III

Chromatographic analysis

The amino-acid composition of culture fractions was investigated by paper chromatography as suggested by CONSDEN, GORDON, AND MARTIN[10] and WOIWOD[2].

One dimensional chromatograms were run on Whatman No. 4 paper with a mixture of *n*-butanol, acetic acid and water (125:30:125 by volume). The same mixture was used as first solvent for two-dimensional chromatography while water-satured phenol was the second solvent. With this solvent combination, methionine cannot be separated from valine, leucine from *iso*leucine nor cystine from $\alpha\varepsilon$-diaminopimelic acid. The last one could be evidenced after hydrogen peroxide treatment. *n*-butanol was purified by distillation, and only the fraction distilling at 116–118° C was used. Phenol was always distilled *in vacuo* over zinc dust. Chromatograms were developed with solvent by descending technique, using a wood cabinet, as suggested by DENT[11], placed in a constant-temperature room at 25° C (± 2°) for 16 hours. The various fractions were placed on the paper using a 0.1 ml pipette, graduated in 0.001 ml divisions, with a capillary right-angled tip. The volumes of culture fractions, which were found to give clear chromatograms, are reported in Table II.

TABLE II

VOLUMES OF THE CULTURE FRACTIONS APPLIED ON THE PAPER FOR CHROMATOGRAPHY

Fractions	*One-dimensional Chromatography ml*	*Two-dimensional Chromatography ml*
Broth filtrate I	0.005	0.015
Cell homogenate II	0.100	0.200
Cell hydrolysate III	0.020	0.050

After irrigation with solvent the paper sheets were dried with a current of warm air. For colour development the dried papers were sprayed with a solution of ninhydrin in chloroform (0.1%), containing 0.1% 2–4–6 collidine. After spraying, the paper was heated in an infrared oven for 5 minutes, then exposed to steam and finally dried again in the oven.

In the two-dimensional chromatograms, the amino acids were identified by comparing the position of the spots with a "map" as indicated by DENT[11]. In the one-dimensional chromatograms, a mixture of known amino acids was run simultaneously on the same sheet of paper for comparison.

References p. 282.

RESULTS

Mycobacterium tuberculosis H37Rv grown on asparagine medium

The results obtained with this strain are shown in Table III. *The hydrolysates* from cells harvested at different days of growth contained most of the common amino acids, but not hydroxyproline. The concentrations of this group of amino acids remained constant during growth. αε-diaminopimelic acid and other ninhydrin-positive substances (R_1, B_1, B_2, and P) were found, but the last four did not remain constant during growth, and if at all, they were present in low concentrations only. The positions of the spots corresponding to these four substances are diagrammatically shown in Fig. 1. Substance R_1 was identified as γ-aminobutyric acid, and substances B_1 and B_2 as methionine sulphone and sulphoxide respectively. The identification was made by running simultaneously a standard substance. Besides, by hydrogen peroxide treatment, the methionine sulphoxide spot disappeared, but not the one corresponding to methionine sulphone. The substance P was not yet identified. These substances could not be found in the other fractions except γ-aminobutyric acid which was constantly found in cell homogenates.

TABLE III

AMINO-ACID COMPOSITION OF FRACTION I, II, III FROM *M. tuberculosis* H37Rv GROWN FOR VARYING DAYS ON ASPARAGINE MEDIUM

	Culture filtrate I					*Cell homogenate II*					*Cell hydrolysate III*				
	Days of growth					*Days of growth*					*Days of growth*				
	7	14	21	28	35	7	14	21	28	35	7	14	21	28	35
Aspartic acid	+	+	+	+	+	—	—	+	+	+	+	+	+	+	+
Glutamic acid	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Asparagine	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—
Lysine	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+
Histidine	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+
Arginine	—	—	—	—	+	—	—	—	—	—	+	+	+	+	+
Alanine	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenylalanine	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+
Glycine	—	—	+	+	+	—	±	+	+	+	+	+	+	+	+
Leucine-*iso*leucine	—	—	±	+	+	—	—	±	+	+	+	+	+	+	+
Methionine-valine	—	—	±	±	+	—	—	±	±	+	+	+	+	+	+
Proline	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+
Serine	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+
Tyrosine	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+
Threonine	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+
Cystine	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+
Cysteic acid	—	—	—	—	—	—	—	—	—	—	+	—	—	+	+
αε-diaminopimelic acid	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+
Polypeptide R	—	—	—	—	—	+	+	+	+	+	—	—	—	—	—
γ-aminobutyric acid	—	—	—	—	—	+	+	+	+	+	—	+	—	±	—
Methionine sulphone	—	—	—	—	—	—	—	—	—	—	+	+	+	+	—
Methionine sulphoxide	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+
Amino acid P	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—

\+ = presence of the amino acid in both one- and two-dimensional chromatograms
± = presence of the amino acid only in one-dimensional chromatograms
— = absence of the amino acid in both chromatograms.

References p. 282.

In the *cell homogenates*, glutamic acid, lysine, alanine, γ-aminobutyric acid and the substance R were constantly present.

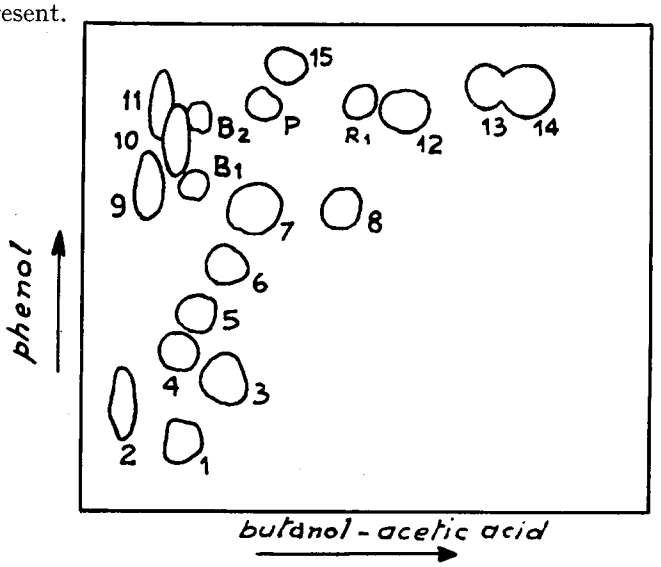

Fig. 1. Diagram showing the position of the spots from cell hydrolysates in two-dimensional chromatograms. Solvents, *n*-butanol-acetic + phenol. 1. Aspartic acid, 2. cystine-αε-diaminopimelic acid, 3. glutamic acid, 4. serine, 5. glycine, 6. threonine, 7. alanine, 8. tyrosine, 9. lysine, 10. arginine, 11. histidine, 12. methionine-valine, 13. phenylalanine, 14. leucine-*iso*-leucine, 15. proline, R_1. γ-aminobutyric acid, B_1. methionine sulphone, B_2. methionine sulphoxide, P. unidentified.

The comparison between Fig. 2 (chromatograms of the homogenate from cells after 7 days' growth) and Fig. 3 (chromatograms of the homogenate from cells after 35 days' growth) clearly reveals that the alanine concentration increases during growth, and the concentration of the substance R decreases but only during the last days of growth.

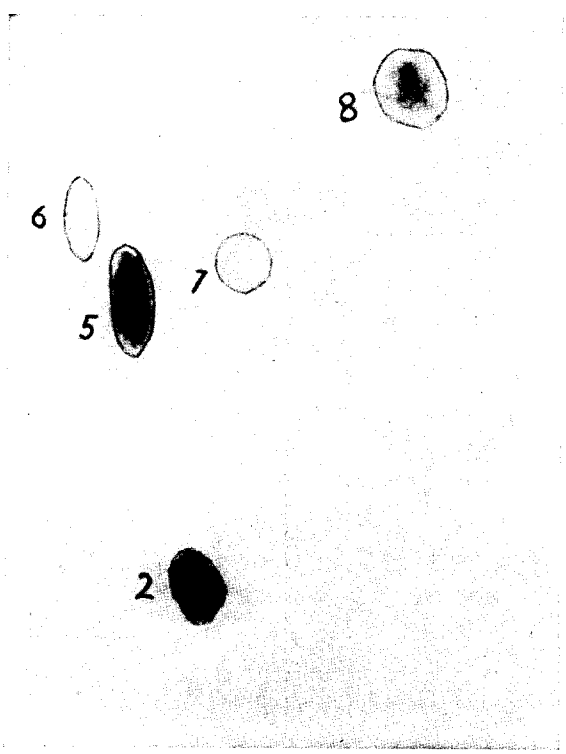

Fig. 2. 7-day cell homogenate, from strain H37Rv grown on asparagine medium, run on two-dimensional chromatogram. Solvents, *n*-butanol-acetic acid + phenol. 2. glutamic acid, 5. polypeptide R, 6. lysine, 7. alanine, 8. γ-aminobutyric acid.

This substance R deserves a particular attention: it is noteworthy that it has been constantly found in the homogenates of all *Mycobacterium* strains investigated. Further, it has a polypeptide structure as has been revealed by our preliminary studies. Indeed, the spot corresponding to this substance (Fig. 2) disappeared after hydrolysis, while other spots were revealed in correspondence to the following amino acids: aspartic acid, leucine-*iso*-leucine, methionine-valine, serine, glycine, threonine, cysteic acid, and

References p. 282.

another unidentified substance B_r, and the colour strength of the spots corresponding to glutamic acid and alanine increased (Fig. 4).

The following amino acids were present after further growth: aspartic acid, glycine methionine-valine, leucine-*iso*leucine, and later serine (Fig. 3).

The chromatographic analysis of the *culture filtrates* showed, as expected, that the asparagine concentration decreases during growth. As for the other amino acids, the results corresponded closely enough to those obtained with the homogenate; only polypeptide R and γ-aminobutyric acid were not present in this fraction. Aspartic acid, glutamic acid and alanine appeared in higher concentration. These last two substances increased during growth.

Mycobacterium tuberculosis H37Rv grown on NH_4Cl medium

When comparing the results obtained with H37Rv strain, grown on YOUMANS medium, to the one obtained with the same strain grown on a similar medium, modified by substitution of asparagine with NH_4Cl (Table IV), no significant variation was observed in the amino-acid composition of the *cell hydrolysate.*

TABLE IV

AMINO-ACID COMPOSITION OF FRACTION I, II, III FROM *M. tuberculosis* H37Rv GROWN FOR VARYING DAYS ON NH_4Cl MEDIUM

	Culture filtrate I						*Cell homogenate II*						*Cell hydrolysate III*					
	Days of growth						*Days of growth*						*Days of growth*					
	9	16	21	28	35	47	9	16	21	28	35	47	9	16	21	28	35	47
Aspartic acid	—	—	—	—	—	—	—	—	—	—	+	—	+	+	+	+	+	+
Glutamic acid	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+
Asparagine	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Lysine	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+
Histidine	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+
Arginine	—	—	—	—	—	+	—	—	—	—	—	—	+	+	+	+	+	+
Alanine	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenylalanine	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+
Glycine	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+
Leucine-*iso*leucine	—	—	—	—	—	+	—	—	—	±	—	—	+	+	+	+	+	+
Methionine-valine	—	—	—	—	—	±	—	—	—	±	—	±	+	+	+	+	+	+
Proline	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+
Serine	—	—	—	—	—	—	—	—	—	—	+	—	+	+	+	+	+	+
Tyrosine	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+
Threonine	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+
Cystine	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+
Cysteic acid	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	+	—	—
αε-diaminopimelic acid	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+
Polypeptide R	—	—	—	—	—	—	+	+	+	+	+	+	—	—	—	—	—	—
γ-aminobutyric acid	—	—	—	—	—	—	+	+	+	+	+	+	—	—	—	+	+	+
Methionine sulphone	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	+	+	+
Methionine sulphoxide	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	+	+	+
Amino acid P	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—	—	—

+ = presence of the amino acid in both one- and two-dimensional chromatograms
± = presence of the amino acid only in one-dimensional chromatograms
— = absence of the amino acid in both chromatograms.

References p. 282.

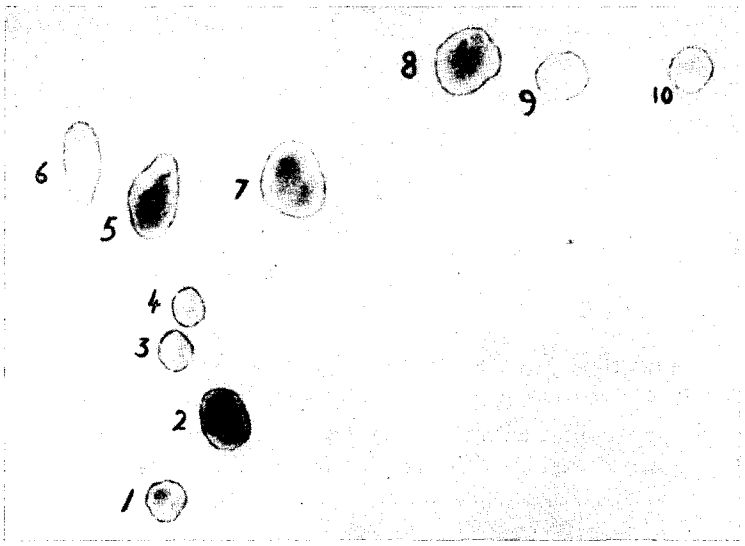

Fig. 3. 35-day cell homogenate, from strain H37Rv grown on asparagine medium, run on two-dimensional chromatogram. Solvents, *n*-butanol-acetic acid + phenol. 1. aspartic acid, 2. glutamic acid, 3. serine, 4. glycine, 5. polypeptide R, 6. lysine, 7. alanine, 8. γ-aminobutyric acid, 9. methionine-valine, 10. leucine-*iso*leucine.

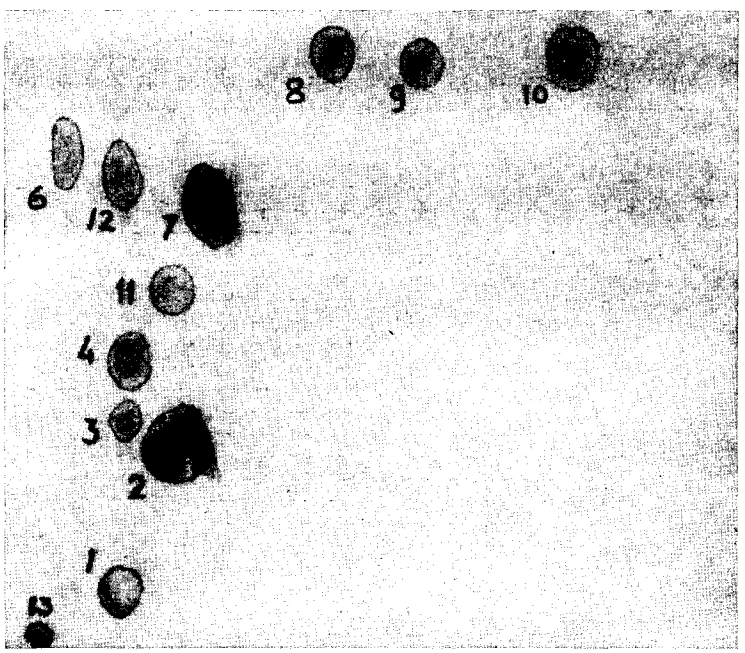

Fig. 4. Acid hydrolysate of 7-day cell homogenate, from strain H37Rv grown on asparagine medium, run on two-dimensional chromatogram. Solvents, *n*-butanol-acetic acid + phenol. 1. aspartic acid, 2. glutamic acid, 3. serine, 4. glycine, 6. lysine, 7. alanine, 8. γ-aminobutyric acid, 9. methionine-valine, 10. leucine-*iso*leucine, 11. threonine, 12. unidentified substance B_r, 13. cysteic acid.

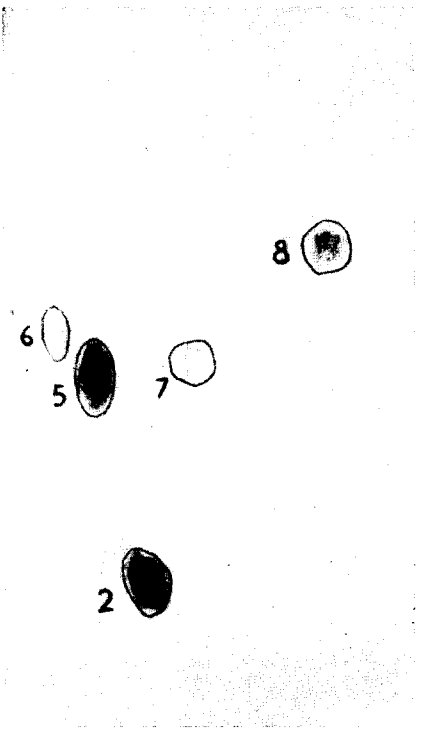

Fig. 5. 9-day's cell homogenate, from strain H37Rv grown on NH_4Cl medium, run on two-dimensional chromatogram. Solvents, *n*-butanol-acetic acid + phenol, 2. glutamic acid, 5. polypeptide R, 6. lysine, 7. alanine, 8. γ-aminobutyric acid.

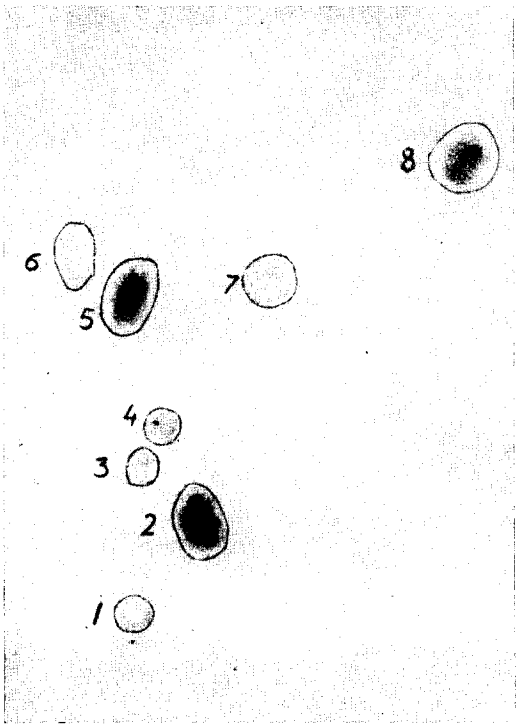

Fig. 6. 35-day cell homogenate, from strain H37Rv grown on NH_4Cl medium, run on two-dimensional chromatogram. Solvents, *n*-butanol-acetic acid + phenol. 1. aspartic acid, 2. glutamic acid, 3. serine, 4. glycine, 5. polypeptide R, 6. lysine, 7. alanine, 8. γ-aminobutyric acid.

As for the *homogenate*, qualitative differences were not observed, in relation to the homogenate of cells grown on asparagine medium, except that the alanine concentration remained constant (Fig. 5 and Fig. 6) while it increased in the asparagine medium. Generally, the amino-acid concentration was lower, but for glutamic acid and polypeptide R.

In the *culture filtrate*, no amino acid was detectable during the first 35 days' growth, but, in the chromatograms of filtrates from cultures after 47 days' growth, glutamic acid, alanine, lysine, arginine, valine-methionine, and leucine-*iso*leucine were found present in very low concentrations. Meanwhile, the concentration of the free amino acids of the cells decreased.

Comparison between M. tuberculosis H37Rv and other five strains

Amino-acid compositions of the three fractions from five different *Mycobacterium* strains, after 7, 14, 21, 28, 35 days' growth, were compared with the amino-acid composition of the strain H37Rv described above. All these strains were grown on YOUMANS medium (asparagine 0.5%), under the same culture conditions.

As for the amino-acid composition of the *cell hydrolysate*, no difference was observed

References p. 282.

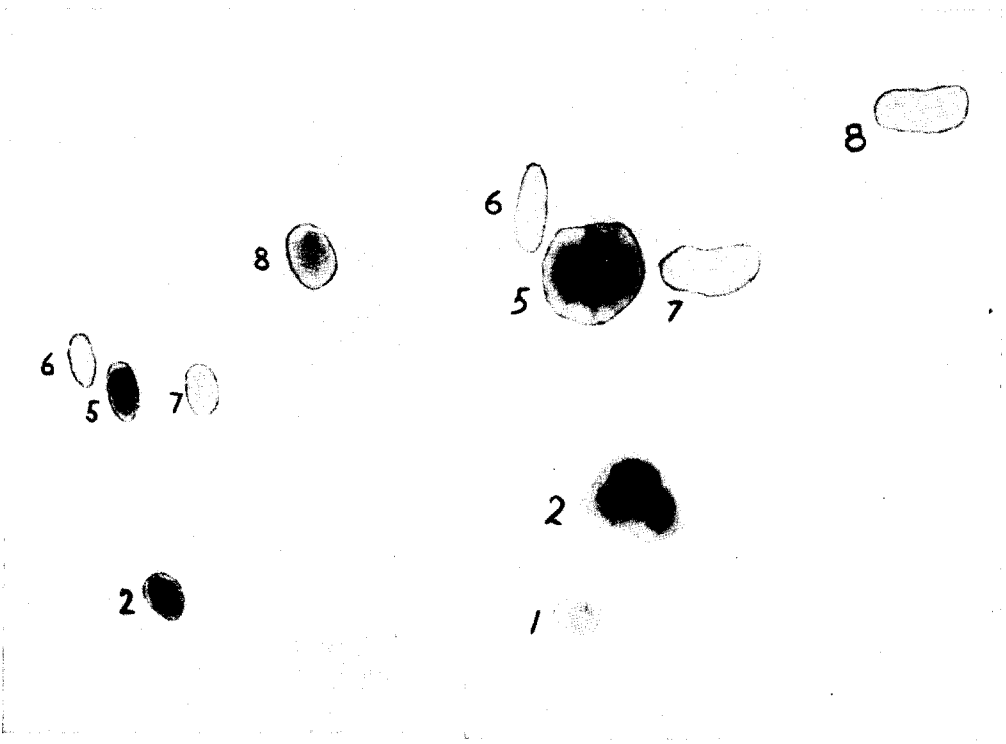

Fig. 7. 14-day cell homogenate, from strain H37Rv grown on asparagine medium, run on two-dimensional chromatogram. Solvents, *n*-butanol-acetic acid + phenol. 2. glutamic acid, 5. polypeptide R, 6. lysine, 7. alanine, 8. γ-aminobutyric acid.

Fig. 8. 14-day cell homogenate, from strain H37Ra grown on asparagine medium, run on two-dimensional chromatogram. Solvents, *n*-butanol-acetic acid + phenol. 1. aspartic acid, 2. glutamic acid, 5. polypeptide R, 6. lysine, 7. alanine, 8. γ-aminobutyric acid.

for the group of the common amino acids. $\alpha\varepsilon$-diaminopimelic acid could be evidenced in cell hydrolysates of all strains. Since the four other substances, γ-aminobutyric acid, methionine sulphone, methionine sulphoxide and substance P, if present, could be found only in low concentrations approaching the sensitivity limits of the analytical method, the evaluation of the differences was difficult. The only significant difference seems to be the constant absence of γ-aminobutyric acid from the cell hydrolysate of *M. tuberculosis* var. *bovis* P: this result is in agreement with the one obtained with the homogenate.

The results obtained with the chromatographic analysis of the *cell homogenate* do not show qualitative differences in the amino-acid composition of this fraction from the six strains studied.

The most outstanding observation is that polypeptide R was always present in the cell homogenate of all the strains considered. Though the quantitative estimation by chromatographic method is far from accurate, certain differences were found between strain H37Ra and the others, particularly after the 14th day of growth. Comparing Fig. 7 (cell homogenate of H37Rv strain after 14 days' growth) with Fig. 8 (cell homo-

References p. 282.

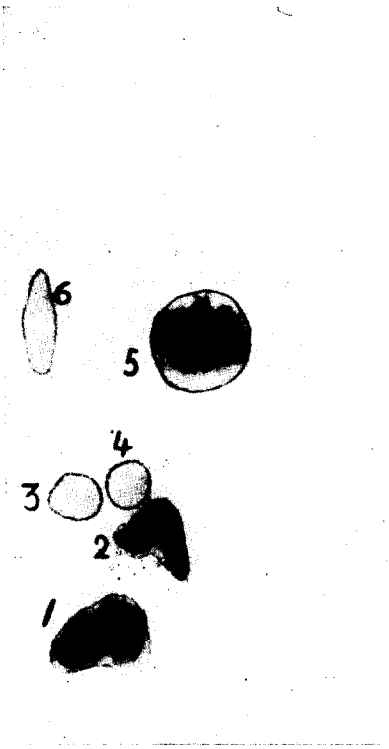

Fig. 9. 21-day culture filtrate, from strain H37Rv grown on asparagine medium, run on two-dimensional chromatogram. Solvents, *n*-butanol-acetic acid + phenol. 1. aspartic acid, 2. glutamic acid, 3. asparagine, 4. glycine, 5. alanine, 6. lysine.

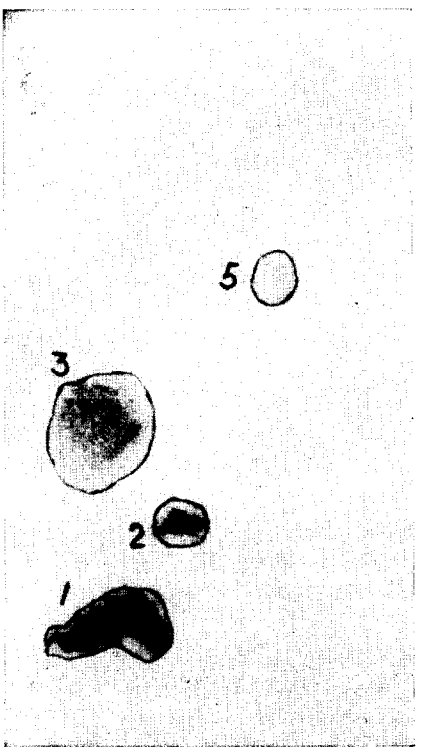

Fig. 10. 21-day culture filtrate, from strain H37Ra grown on asparagine medium, run on two-dimensional chromatogram. Solvents, *n*-butanol-acetic acid + phenol. 1. aspartic acid, 2. glutamic acid, 3. asparagine, 5. alanine.

genate of H37Ra strain after 14 days' growth), it can be seen that the glutamic acid and, more evidently, polypeptide R concentrations are higher for H37Ra homogenate. Constantly, alanine remained in low concentration throughout growth. As for the *M. tuberculosis* var. *bovis* P strain, it could be noted that γ-aminobutyric acid, which remained in constant concentration in the homogenate from the other strains, decreased during growth, and thoroughly disappeared after 35 days' growth.

As for the *culture filtrates* of the six strains the only qualitative difference concerned the strain H37Ra. In the culture filtrates from this strain the group of amino acid which appeared later during growth in the broth from H37Rv (lysine, arginine, glycine, leucine-*iso*leucine, methionine-valine) was not detected.

Interesting quantitative differences could be observed within the limits of the method, with regard to this strain. Comparing Fig. 9 (culture filtrate from H37Rv strain after 21 days' growth) with Fig. 10 (culture filtrate from H37Ra strain after 21 days' growth), it can be seen that in the latter glutamic acid and alanine are present in low concentrations. Alanine remained constantly in low concentration during growth, while it increased in the culture filtrates from the other strains. In the filtrate of H37Ra strain an evident decrease of asparagine was not detected (Fig. 10). *M. avium* Panisset

References p. 282.

was the only strain in which a similar phenomenon could be observed, though it was less remarkable. In the culture filtrate of this strain, a weak spot was found after 28 days' growth; its R_F corresponded to that of γ-aminobutyric acid present in the homogenate.

CONCLUSIONS

The method (paper chromatography) selected for the study of the amino-acid composition of *Mycobacteria*, in spite of its limitations as a quantitative test, permitted certain general observations, interesting enough to encourage the extension of the work presented. Tubercle bacilli, owing to the slowness of their growth, are particularly suitable for a study in which the amino-acid composition as a function of the time period of growth might have a particular importance.

The amino-acid composition of the different fractions from cultures in asparagine medium was relatively similar, from a qualitative point of view, in all the *Mycobacterium* strains tested. Quantitative differences could be estimated only with approximation by the method employed. Though these seem to be of a limited extent they will be evaluated by more suitable quantitative methods. Some difference could be noted in the amino-acid concentration in cultures of *M. tuberculosis* H37Ra. In the homogenates of this strain, alanine and the other amino acids are in lower concentration. But glutamic acid and particularly polypeptide R are in higher concentration than in the homogenates from the other strains. Also in the culture filtrates all the amino acids remain in low concentrations and the asparagine utilization appears less evident in the chromatograms.

This picture shows some analogy with the one described in strain H37Rv, grown on NH_4Cl medium. This observation can be explained by a slow metabolism in both cases.

In the hydrolysate of the strains tested all the common amino acids could be detected in higher concentrations except hydroxyproline. $\alpha\varepsilon$-diaminopimelic acid was found constantly present, in agreement with WORK[12]. Four more ninhydrin-positive substances were found at much lower concentrations.

Three of these substances were identified as γ-aminobutyric acid, methionine sulphone and methionine sulphoxide. The inconstant presence of the last two substances and of cysteic acid can be explained by considering them as products derived from other amino acids (methionine and cystine) during technical manipulation. The substance P has not yet been identified.

The γ-aminobutyric acid spot was not constantly found in the chromatograms of cell hydrolysates, probably because this substance is present only in the free state inside the cells and is not combined in the protein material; hence, in cell hydrolysates its concentration is much lower than that of other amino acids, at the sensitivity limits of ninhydrin reaction.

Our study indicates that the analysis of the homogenates derived from *Mycobacterium* strains can give more valuable information on the amino-acid metabolism than the other fractions considered. In that fraction glutamic acid, lysine, alanine, γ-aminobutyric acid, polypeptide R, aspartic acid, glycine, leucine-*iso*leucine, methionine-valine and serine, were detected. The first five appear as free substances inside the cell during early growth. All these substances are present even when the sole source of nitrogen is an ammonium salt. In this case the concentration is lower, except for

References p. 282.

glutamic acid and polypeptide R. Some analogy could be observed between the composition of the cell homogenate and of the culture filtrate. The most striking difference is the constant absence of polypeptide R and γ-aminobutyric acid from the filtrates. The latter amino acid was found only in the filtrates from *M. avium*.

The constant presence of polypeptide R inside the cell represents an observation worth of further attention. Investigations on the structure of this polypeptide are under way in our laboratory.

ACKNOWLEDGEMENTS

The authors are greatly indebted to Dr E. WORK, London, for samples of $\alpha\varepsilon$-diaminopimelic acid and γ-aminobutyric acid and to Mr W. STEENKEN, Jr., Trudean, N.Y., for the *Mycobacterium* strains H37Rv and H37Ra.

SUMMARY

The amino-acid composition of a number of *Mycobacterium* cultures was investigated by the paper chromatography technique. The chromatographic analysis was carried out at different periods of growth and in different culture fractions; namely, in the culture filtrate, in the cell homogenate and in the cell hydrolysate.

The results obtained for the different strains tested were found relatively similar from a qualitative point of view. Quantitative differences could be estimated with the approximation permitted by the method. In the hydrolysate all the common amino acids could be detected in higher concentrations except hydroxyproline. $\alpha\varepsilon$-diaminopimelic acid was found constantly present. The presence of γ-aminobutyric acid, methionine sulphone and methionine sulphoxide was not found constant.

More interesting results could be obtained from the analysis of the cell homogenate. In that fraction glutamic acid, lysine, alanine, γ-aminobutyric acid, polypeptide R, aspartic acid, glycine, leucine-*iso*leucine, methionine-valine, and serine, were detected.

The first five appeared as free substances inside the cell during early growth and they remained present throughout. Some analogy could be observed between the composition of the cell homogenate and the culture filtrate, but, in the last fraction polypeptide R and γ-aminobutyric acid were absent.

RÉSUMÉ

On a étudié à l'aide de chromatographies sur papier la composition en acides aminés de quelques souches de *Mycobacterium*. Les analyses chromatographiques ont été executées en diverses périodes sur différentes fractions de culture et plus précisément sur le filtrat, sur l'homogénéisat et sur l'hydrolysat des cellules bactériennes.

On a trouvé que les résultats obtenus pour les différentes souches étaient relativement analogues au point de vue qualitatif. On a pu apprécier, dans les limites consenties par la méthode, des différences quantitatives.

Dans les hydrolysats on a pu mettre en évidence tous les acides aminés communs excepté l'hydroxyproline. On a constamment trouvé de l'acide $\alpha\varepsilon$-diaminopimélique, tandis que l'acide γ-aminobutyrique, le sulfone, et le sulfoxyde de la méthionine n'étaient pas toujours présents. On a pu obtenir des résultats plus intéréssants à l'aide de l'analyse de l'homogénéisat. Dans cette fraction on a trouvé l'acide glutamique, la lysine, l'alanine, l'acide γ-aminobutyrique, un polypeptide R, l'acide aspartique, la glycine, le groupe leucine-*iso*leucine, le groupe méthionine-valine et la sérine. Les cinq premiers produits étaient présents dans l'homogénéisat dès les premiers moments du développement et on les y a constamment trouvés.

Il existe une certaine analogie entre les résultats obtenus avec les homogénéisats et ceux obtenus avec les filtrats de culture. Mais dans cette dernière fraction de culture, l'acide γ-aminobutyrique et le polypeptide R n'étaient pas présents.

ZUSAMMENFASSUNG

Es wurde die Zusammensetzung der Aminosäuren einiger Stämme von *Mycobacterium* chromatographisch (Papierchromatographie) untersucht. Die chromatographische Analyse wurde in verschie-

References p. 282.

denen Entwicklungsstadien und bei verschiedenen Kulturfraktionen (Kulturfiltrat, Bakterienhomogenat und Bakterienhydrolysat) durchgeführt.

Aus den Ergebnissen der Analyse der verschiedenen Stämme ging hervor, dass diese qualitativ sehr ähnlich sind. Quantitative Unterschiede konnten geschätzt werden, entsprechend der Genauigkeit dieser Methode. In den Bakterienhydrolysaten wurden alle gewöhnlichen Aminosäuren ausser Oxyprolin, nachgewiesen. Die $\alpha\varepsilon$-Diaminopimelinsäure war immer vorhanden; hingegen waren die γ-Aminobuttersäure und das Methioninsulfon und -sulfoxyd nicht immer zugegen.

Die interessantesten Ergebnisse erhielten wir aus der Analyse der Bakterienhomogenate. Glutaminsäure, Lysin, Alanin, γ-Aminobuttersäure, ein besonderes R Polypeptid, Asparaginsäure, Glyzin, die Leuzin-*Iso*leuzin Gruppe, die Methionin-Valin Gruppe und Serin waren immer vorhanden.

Die ersten fünf Substanzen waren in den Bakterienhomogenaten schon in den Anfangsstadien der Entwicklung in freier Form zugegen, und blieben es auch nachher. Es besteht eine gewisse Ähnlichkeit zwischen den Ergebnissen der Analyse der Bakterienhomogenate und der Kulturfiltrate; nur die γ-Aminobuttersäure und das R Polypeptid waren in den Kulturfiltraten nicht vorhanden.

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Received October 23rd, 1951