A COMPARISON OF THE CHEMICAL COMPOSITION OF MYCOBACTERIUM TUBERCULOSIS MURIS WITH MYCOBACTERIUM TUBERCULOSIS BOVIS (BCG)

George Caldes and Benjamin Prescott

Laboratory of Microbiology
National Institute of Allergy and Infectious Diseases
National Institutes of Health, Bethesda, Maryland 20014

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Summary. Comparative chemical analyses were performed on Mycobacterium tuberculosis bovis (BCG) and M. tuberculosis muris organisms grown under the same conditions. The amino acid contents were similar. The same carbohydrates were found in both organisms. However, the muris bacillus had twice as much glucose as the BCG. The BCG organism contained more lipid, and had four times more material extractable by chloroform, than the muris bacillus.

Two strains of Mycobacterium tuberculosis are presently being used as effective vaccines (6,7). Bacillus Calmette Guerin (BCG) is an attentuated strain of a formerly pathogenic bovine bacillus. Mycobacterium tuberculosis muris (the vole bacillus) is a natural strain which is normally pathogenic only to a small rodent, the vole, (Microtis agrestis). Sula, Galliova, and Scrivanova (12) found much greater local tissue destruction from BCG inoculum than from the murine inoculum. On the other hand, the murine inoculum is more virulent, and has caused localized tuberculosis in human subjects (6,10). Sula et al (12) did comparative studies on the lipid content of BCG and a strain of muris bacillus, but the two organisms were grown in different media. To the authors' knowledge, no other chemical studies involving the muris bacillus have been made. The present study concerns the comparative amino acid, carbohydrate, and lipid content of the two organisms grown under identical conditions. Knowledge of the chemical composition of BCG and muris organisms, and comparison of these with other studies and studies done on other strains may help explain some of the characteristics of tuberculosis vaccines.

Methods

Cultural Conditions. The organisms were grown on modified Long's medium (4). This medium was found suitable for obtaining rapid and heavy growth. One hundred ml portions of the medium were dispensed into 250 ml Erlenmeyer flasks, then sterilized by autoclaving for 20 min. at 115°. The seed cultures were grown on Jensen's egg slants. The flasks were seeded by transferring a small section of growth from the slant to each flask. After incubating for 2 to 4 weeks at 37°, the cells were harvested by swirling the flasks, and quickly pouring the contents onto a washed No. 41 Whatman filter paper into a Buchner funnel. The organisms were washed twice with distilled water, and dried at 100° for 48 h.

Lipid Extraction. One gram of dried organisms was extracted in a Sohxlet apparatus successively with 50 ml of 7 solvents of increasing polarity, 12 h per solvent, as follows: chloroform, chloroform-ether 1:1, ether, ether-ethanol 1:1, ether-ethanol 1:2, ethanol, and 70% ethanol.

Amino Acid Analysis. Three hundred mg of defatted organisms were refluxed in 100 ml of 6N HCl at 120-130° for 18 h. The acid was evaporated under vacuum, the residue suspended in warm water, filtered, and evaporated to dryness. The dry residue was dissolved in 20 ml of 5% isopropanol. Fifty ml samples were chromatographed on Whatman No. 3 paper according to the method of Wolfe (14), substituting the ninhydrin reagent of Barrolier (1) for color development. The colored spots and adjacent areas of paper for blanks were cut out and leached in 5-20 ml of methanol for 3-5 h. The intensity of the red color was measured at 525 nm wave length in a Fisher electrophotometer, and the amino acid content calculated by reference to standard curves. Four or more replicate readings were obtained with this method, with average deviations from the mean of less than 10%. Proline was determined by the method of Chinard (2). The yellow proline spot was cut out and treated with Chinard's reagent, and the color intensity of the resulting red solution measured at 525 nm. Tryptophan was determined by means of the method of Roth and Shuster (11).

Ammonia was estimated from the ammonia content of the amono acid hydrolysate by the micro-Kjeldahl method.

Carbohydrate Analysis. Carbohydrates were determined by hydrolysing 10-15 mg of bacilli at 105° for 3 h in 4 ml of 2N sulfuric acid in a sealed

TABLE I

COMPARISON OF THE AMINO ACID CONTENT OF DEFATTED BACILLUS CALMETTE GUERIN WITH THE MYCOBACTERIUM TUBERCULOSIS MURIS (VOLE BACILLUS)

Amino Acid	Vole bacillus	BCG
Alanine	5.1 %	5.7 %
Arginine	4.6	4.9
Aspartic Acid	4.6	5.9
Cystine + Cysteine	1.9	1.7
Glutamic Acid	7.0	7.6
Glycine	3.2	3.2
Histidine	1.1	1.2
Isoleucine	1.8	1.7
Leucine	3.4	3.6
Lysine	2.4	3.2
Methionine	0.5	0.8
Phenylalanine	1.3	1.0
Proline	3.7	3.6
Serine	1.8	1.9
Threonine	2.8	2.9
Tryptophan	1.8	1.9
Tyrosine	1.1	1.6
Valine	4.0	3.4
Total Amino Acids (% of dry weight)	52.1 %	55.8 %
Ammonia	2.0 %	2.1 %
Humin Nitrogen	0.2 %	0.2 %

tube, removing the sulfuric acid by passing the hydrolysate through a column of Amberlite IR-45 ion exchange resin (acetate form) 15 cm high by 1 cm in diameter. The solution containing the carbohydrate was lyophilized, the residue taken up in 2 ml of water and chromatographed on Whatman No. 1 paper according to the method of Colombo et al (3). The carbohydrate spots were leached with 60% ethanol, and the amount of each carbohydrate estimated by comparing the color intensities of the solutions at 420 nm with the color intensities from standard solutions chromatographed simultaniously.

Hexosamines were determined with the Levvy modification (8) of the Elson-Morgan method on a hydrolysate made by heating 10 mg of whole organisms in a sealed tube with 4 ml 8N HCl 4 h at 110°.

Results and Discussion

The amino acid patterns of the paper chromatograms and the amounts of each acid from both organisms were similar (Table 1). Seventeen known alpha amino acids were found, plus a small unidentified spot in the same place on each chromatogram. Ginsburg et al (5) did amino acid analyses on ten strains of mycobacteria, and found them very similar, except for one human virulent and one "cord factor" producing strain which were 35-60% higher in glutamic acid than the average of the ten. He suggested that high glutamic acid content may be associated with virulence. Long (9) describes other studies in which glutamic acid is associated with virulence. In our study the vole bacillus was not of higher glutamic acid content than the BCG, although it is pathogenic to the vole, and is considered more virulent to humans than BCG. The BCG of Ginsberg's series was of average glutamic acid content, and the glutamic acid content was similar to that of this study.

Qualitatively, the same carbohydrates were found in both organisms, with arabinose present in larger amount than the other sugars. The combined amounts were similar, but the vole organism contained twice the amount of glucose (Table 2). Glucose is found in tubercle bacilli in the form of glycogen, and as the disaccharide, trehalose, which replaces glycerol in

TABLE 2

CARBOHYDRATE ANALYSIS

	Vole Bacillus	Bacillus Calmette Guerin
Arabinose	4.4%	4.9%
Galactose	2.6	2.4
Glucose	2.7	1.1
Mannose	1.9	2.4
Ribose	Detectable	Detectable
Total Sugars	11.6%	10.8%
Hexosamine	2.2	1.9

the neutral fats. Trehalose is also a component of the cording factor, which is associated with virulence (9,15). Analyses for glucose on lipid fractions, or fractions of other components may help explain the manifestation of virulence.

TABLE 3

LIPID EXTRACTABLES

Solvent	Vole Organism	Bacillus Calmette Guerin
Chloroform	4.8 %	20.3 %
Chloroform-ether 1:1	0.3	1.3
Ether	0.0	0.4
Ether-ethanol 1:1	0.3	1.2
Ether-ethanol	2.3	1.8
Ethanol	8.6	2.1
70% Ethanol	7.5	4.8
Total %	23.8	31.9

The vole bacillus contained less lipid and had greater polarity than the BCG lipid (Table 3). A comparison of the elemental analyses of the two organisms before and after defatting indicated a larger amount of phosphorus in the lipid of the vole bacillus, since the percent phosphorus of the defatted vole organism was less than before lipid extraction while the reverse was true of the BCG. The chloroform extractables of the BCG contained 0.4% phosphorus, while that of the muris organism contained 0.8%. Sula et al (12) did comparative studies of the lipid content of a muris strain, M.P.-Prague, which had been grown on synthetic medium, with two BCG strains grown on Sauton's medium, and found much less lipid extractable with chloroform from the muris strain than from the other strains. The chloroform soluble lipids appear to be necessary for the production of the characteristic tuberculosis allergy (9,15). In this study four times more material was extractable by chloroform from BCG than from muris bacillus grown under the same conditions. According to Wells and Wylie (13) ten or more times as much BCG inoculum by weight is needed to produce the same immunity as that produced by the vole bacillus. It may be that the material extracted by chloroform interferes with the reactions that produce immunity. Selection of low wax containing mutants or growth conditions that inhibit wax production may then yield inoculums of greater efficiency as well as of milder allergic response.

References

1. Barrolier, J., Naturwissenschaften 14, 416 (1955).
2. Chinard, F. P., J. Biol. Chem. 199, 91 (1952).
3. Colombo, D., Corbetta, D., Pirotta, A., Ruffini, C., and Sartori, A. J. of Chromatog. 3, 343 (1960).
4. Drea, W. F., J. Bact. 39, 197 (1940).
5. Ginsburg, B., Lovett, S. L., and Dunn, M. S., Archives of Biochem. and Biophys. 60, 164 (1956).
6. Hart, P. D., Sutherland, I., and Thomas, J., Tubercle 48, 201 (1967).
7. Irvine, K. N., "BCG and Vole Vaccination" 2nd edition, Waterlow and Sons, London (1957).
8. Levvy, G. A., and McAllen, A., Biochem. J., 73, 127 (1959).
9. Long, E. R., "The Chemistry and Chemotherapy of Tuberculosis" pgs 101, 145-146 The Williams and Wilkins Co., Baltimore, Md. (1958).

10. Maguire, A., British Medical Journal 527 (1969).
11. Roth, H., and Shuster, P. H., Angew. Chemie 52, 149 (1939).
12. Sula, L., Galliova, J., and Scrivanova, R., Bull. World Health Organization 37, 953 (1967).
13. Wells, A. Q., and Wylie, I. A. H., British Med. Bull. 10, 96 (1954).
14. Wolfe, M., Biochimica et Biophysica Acta 23, 186 (1957).
15. Youmans, G. P., and Youman's A. S., C. T. in Microbiology 48, 133 (1969).