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Multiple catalases of the mycobacteria

Catalase activity was demonstrated in mycobacteria as early as 1897[1], but it was not until 1954 that MIDDLEBROOK *et al.*[2] noted the direct relationship between catalase activity of *Mycobacterium tuberculosis*, its isoniazid resistance, and virulence for the guinea pig. Examination of catalase activity in mycobacteria suggests the presence of two enzymes; one heat stable and the other heat labile[3]. Although several enzymes of mammalian catalases can be distinguished immunologically[4], only minor differences in molecular weight or isoelectric point have been demonstrated[5,6]. Similar minor differences in electrophoretic mobility in agar gel were observed in mycobacterial catalases[7,8]. It is possible that a better analytical technic might help to separate the mycobacterial catalases and reveal any species-associated differences.

Materials and methods

Preparation of acrylamide gel. 5 % acrylamide with a 2.5 % cross linkage was prepared in Tris buffer pH 9.5 following the procedure of ORNSTEIN AND DAVIS[9]. The gel was placed in a 7 × 100 mm glass tube producing a column of acrylamide 90 mm long. A separator and sample gels were not used.

Cultures used and preparation of enzyme extract. The following cultures were used: *M. terrae* (1456–66), *M. triviale* (729–68), and *M. smegmatis* (BC 101). Cultures of these acid-fast bacilli were grown in Roux bottles containing 150 ml of Dubos-Davis broth enriched with glucose containing Tween 80. Following incubation for 7 days, the cultures were checked for contamination, centrifuged, washed twice with saline, and shaken with 16–18 mesh plastic beads in a Braun MSK homogenizer. Cell wall and other cellular debris were removed by centrifugation at 15000 × *g* for 30 min. Supernatant and cell wall were stored separately at —20° for further use.

Electrophoresis. 0.1 ml of the cell-free extract, heated at 68° for 20 min or unheated, was diluted with 0.3 ml of a 20 % sucrose solution containing Bromphenol Blue. The appropriate number of tubes of acrylamide gel were overlayed with a 0.05 ml of this mixture and run in the cold for 30 min at 1 mA per tube, followed by $2^1/_2$ h at 5 mA per tube. Diethyl barbiturate buffer pH 8.6 was used in the reservoirs.

Staining for enzyme activity. The gel was removed from the glass tubes and placed in 4 % starch in phosphate buffer pH 7 for 30 sec, washed with running water, and placed in a 1 % solution of potassium iodine acidified with acetic acid. The tubes were read within 15 min and the actual migration distance and relative mobility (percent of the distance migrated by the fastest band in culture 729–68) was recorded.

Results

The slower moving, partially heat sensitive band seen in the extract from *M. triviale* (Fig. 1; tube 1, 2) was considered to have a relative migration of 88 % and is heat stable. *M. terrae* catalase was differentiated into two bands as well, but with mobilities of 94 % and 76 % respectively (Fig. 1; tube 3, 4). Both bands were heat stable. *M. smegmatis* (Fig. 1; tube 5, 6), on the other hand, has only one heat sensitive catalase which is slower than any of the other four enzymes. Bovine liver catalase (Fig. 2) is much slower than the slowest observed mycobacterial enzyme. Both the

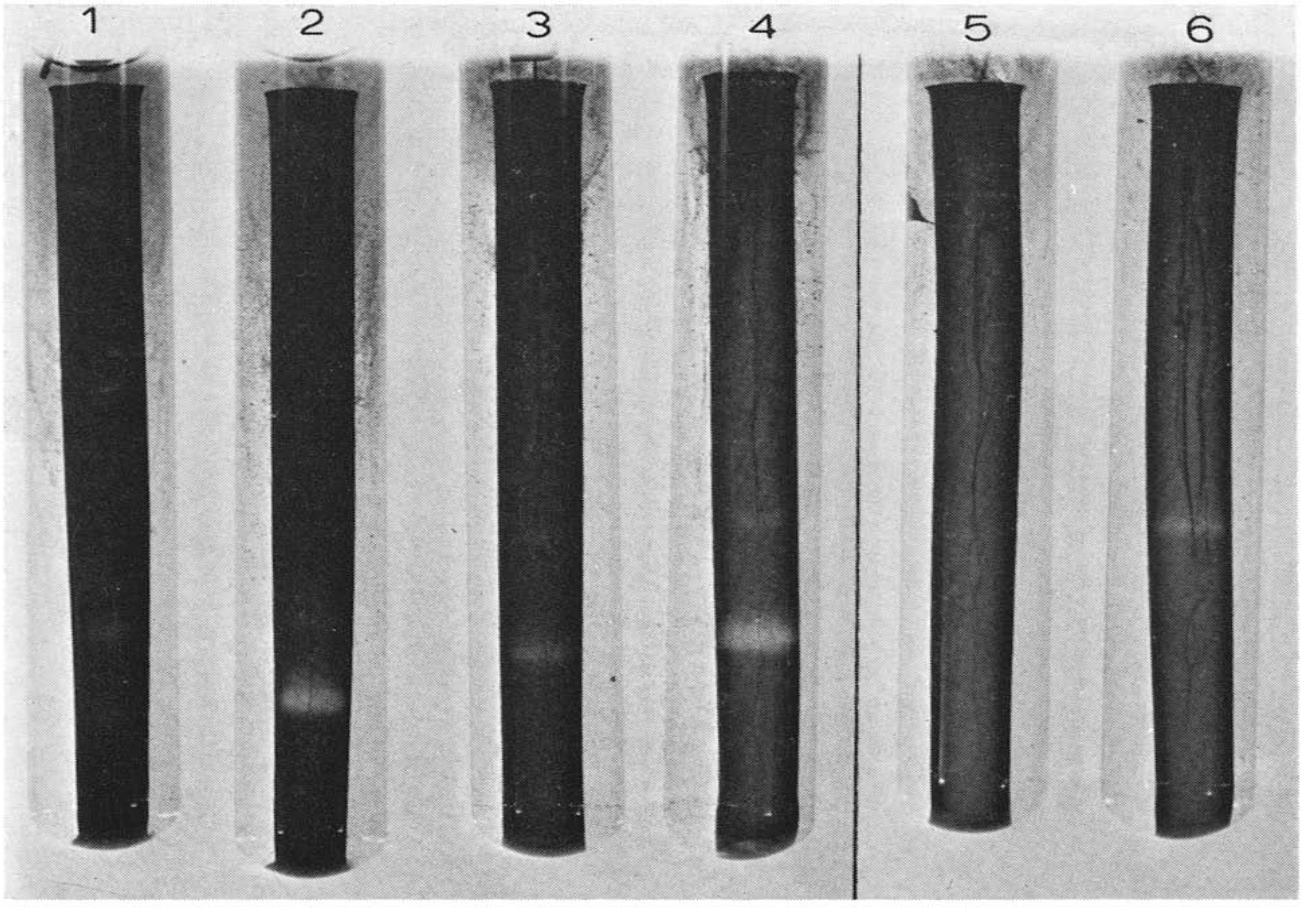

Fig. 1. All six tubes are from one run in 5 % acrylamide for 2 1/2 h. The extracts in the first two tubes on the left are from *M. triviale*, the middle tube from *M. terrae* and the two on the right from *M. smegmatis*. The extracts in tubes 1, 3, 5 were heated at 68° for 20 min.

bovine and mycobacterial enzymes were inhibited by 10^{-4} *M* sodium azide[10] and horseradish peroxidase did not produce any bands in this system. Fig. 3 is a diagrammatic representation of the various bands of enzymes found in these mycobacteria.

Discussion

Although catalase is one of the oldest enzymes known, little has been reported concerning the presence of isoenzymes. The 240000 molecular weight mammalian catalase has been found to be a tetramer of 60000 molecular weight polypeptide chains and has been reported to be present in multiple molecular forms[6]. Mycobacterial catalases, on the other hand, have not been thoroughly investigated. To date, two catalases have been reported in mycobacteria; a heat labile and a heat stable enzyme[3]. The heat labile enzyme is inactivated by heating at 68° for 20 min and has been shown to be related, in *M. tuberculosis*, to INH resistance and pathogenicity for the guinea pig[2]. Although these observations suggested a multiplicity of molecules with catalase activity, further characterization was not fruitful. BARTHOLOMEW[7], using this staining technic and agar gel electrophoresis, demonstrated two bands of enzyme activity and eliminated the possibility that the bands could have been due

to a peroxidase or an amylase. NAKAYAMA[8] failed to detect enough differences between mycobacterial catalases to make them useful in classification or identification. In the present work, a more refined method, acrylamide gel electrophoresis, was used. Acrylamide gel has the advantage of separating molecules on the basis of both electrical charge and molecular weight. By using a low concentration of acrylamide gel, it was possible to identify five different catalases within the three species of mycobacteria studied. One is heat sensitive, a second is partially inactivated by heat, and

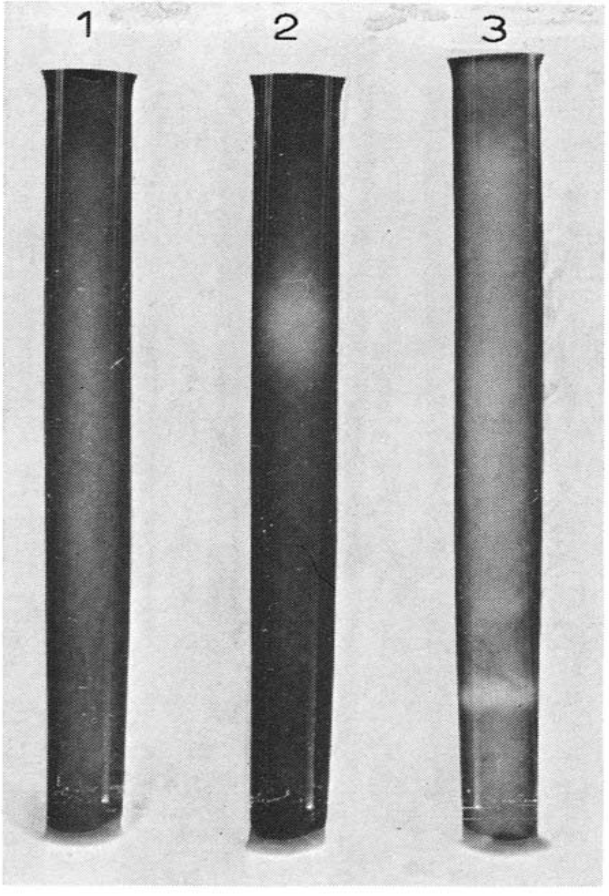

Fig. 2. All three tubes are from one run in 5% acrylamide for $2^1/_2$ h. The material in the tube on the left contains 50 units of bovine liver catalase heated at 68° for 20 min. The middle tube contains unheated bovine liver catalase and the tube on the right contains unheated *M. triviale* extract.

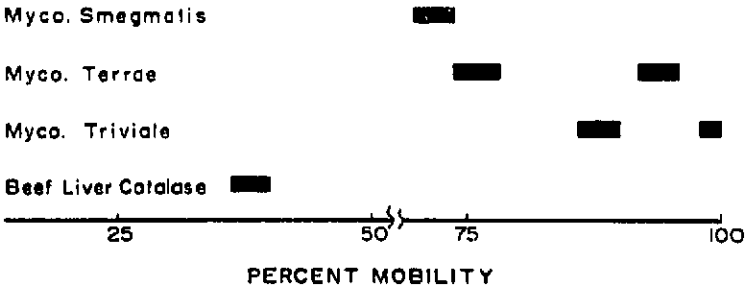

Fig. 3 Diagrammatic representation of the relative mobilities of the enzymes studied. Each box on the graph represents the range of results with a minimum of ten runs.

three are heat resistant. There is no relationship between migration and heat sensitivity.

These findings indicate that mycobacterial catalases are present in multiple molecular forms. All of these forms appear to have a lower molecular weight than the previously studied mammalian and bacterial catalases. Studies are currently underway to determine if these zymograms can be used for classification of different mycobacteria.

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1 M. HAHN, *Muenchen. Med. Wochenschr.*, 48 (1897) 1344.
2 G. MIDDLEBROOK, M. L. COHN AND W. B. SCHAEFER, *Amer. Rev. Tuberc.*, 70 (1954) 852.
3 G. P. KUBICA AND G. L. POOL, *Amer. Rev. Resp. Dis.*, 81 (1960) 387.
4 E. T. NISHIMURA, S. N. CARSON AND T. Y. KOBARA, *Arch. Biochem. Biophys.*, 108 (1964) 452.
5 Y. HOKAMA, *Cancer Res.*, 27 (1967) 2300.
6 R. S. HOLMES AND C. J. MASTERS, *Arch. Biochem. Biophys.*, 109 (1965) 196.
7 W. R. BARTHOLOMEW, *Amer. Rev. Resp. Dis.*, 97 (1968) 710.
8 Y. NAKAYAMA, *J. Microbiol.*, 11 (1967) 95.
9 L. ORNSTEIN AND B. J. DAVIS, *Ann. N.Y. Acad. Sci.*, 121 (1964) 404.
10 M. DIXON AND B. C. WEBB, *Enzymes*, Academic Press, New York, London, 1958.

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Separation of isomeric lysine derivatives by ion-exchange chromatography

The biochemical literature contains numerous references to the chemical modification of gelatin[1,2]. The present interest in the reaction between gelatin and N-acetylsulphanilyl chloride was twofold. Firstly, that it should provide information regarding the reactivity of the reagent with this particular protein. In addition, that the acid hydrolysate of the modified gelatin should contain in fair yield Nε-(*p*-aminosulphanilyl)-lysine, a sulphonamide and potentially at least, a useful anti-inflammatory agent.

During the course of the work considerable difficulties were experienced in locating the Nε-(*p*-aminosulphanilyl)-lysine in the hydrolysate when employing the normal column chromatographic procedures of MOORE AND STEIN[3]. To resolve this problem, pure lysine derivatives were prepared for use as model substances. Reaction of L-lysine monohydrochloride with an excess of N-acetylsulphanilyl chloride gave