Virulence and Resistance to Superoxide, Low pH and Hydrogen Peroxide among Strains of *Mycobacterium tuberculosis*

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(Received 10 August 1977; revised 19 September 1977)

Six strains of *Mycobacterium tuberculosis* of different virulence in guinea-pigs were compared with regard to their resistance to low pH, to hydrogen peroxide (H_2O_2) at different pH values and to superoxide ($\cdot\text{O}_2^-$). Low virulence was associated with susceptibility to H_2O_2 in native and isoniazid-resistant strains but not in laboratory-attenuated strain H37Ra. H_2O_2 resistance was only partly related to catalase content. Low virulence was not associated with susceptibility to an acid environment but the tuberculocidal effect of H_2O_2 was significantly increased at low pH. The strains were uniformly resistant to $\cdot\text{O}_2^-$ and contained similar amounts of superoxide dismutase. The implications of these observations are discussed in the context of mechanisms of host defence in tuberculosis.

INTRODUCTION

Strains of *Mycobacterium tuberculosis* can differ widely in virulence when tested in laboratory animals. Low-virulence strains can be classified into three groups according to their origins: (1) strains with low virulence on primary isolation [isolates of tubercle bacilli from tuberculosis patients in India and neighbouring countries are commonly of this type (Mitchison, 1970)]; (2) strains which have become attenuated in virulence by mutation to a high degree of resistance to the chemotherapeutic agent isoniazid (Middlebrook, 1957); (3) strains in which attenuation of virulence has arisen during maintenance of the strains in the laboratory (Dubos, 1955). The explanations of low virulence may be different for each of these three groups of strains and identification of the defects would help to define the major mechanisms of host defence against tubercle bacilli.

In strains of groups 1 and 2, low virulence is manifested in the guinea-pig as a poor ability to cause disease that progresses after the onset of the cell-mediated immune response. Before activation of this host response, differences in virulence are not readily apparent (Middlebrook, 1957; Mitchison *et al.*, 1960). Virulence in *M. tuberculosis* thus appears to depend on an ability to withstand the enhanced bactericidal powers of activated macrophages, the probable final effector cells of antibacterial cell-mediated immunity (Blanden *et al.*, 1976). Studies of strains with low virulence should therefore help to establish how activated macrophages kill micro-organisms more efficiently than normal macrophages. Group 3 includes strains, typified by strain H37Ra, which show a high degree of attenuation of virulence, failing to multiply or multiplying slowly even in non-immunized animals (Dubos, 1955; Alsaadi & Smith, 1973). Studies of such strains might clarify the nature of the host defence mechanisms which operate even in non-immunized animals.

Diverse properties of tubercle bacilli have been suggested as determinants of virulence (Dubos, 1955; Mitchison, Selkon & Lloyd, 1963; Goren, Brokl & Schaefer, 1974*a, b*; Wietzerbin, Lederer & Petit, 1975). Hydrogen peroxide (H_2O_2) is one of the bactericidal

products of macrophages (Klebanoff & Hamon, 1975) and resistance to H_2O_2 is a virulence attribute in *M. tuberculosis* (Mitchison *et al.*, 1963). However, resistance to other anti-bacterial macrophage products such as low pH or superoxide ($\cdot\text{O}_2^-$) may be involved. We have compared strains of *M. tuberculosis* of different native or attenuated virulence with regard to (i) the effect of pH on their survival in the presence and absence of H_2O_2 and (ii) their resistance to $\cdot\text{O}_2^-$. Evidence has been sought that low virulence can be ascribed to different factors in native, isoniazid-resistant and laboratory-attenuated tubercle bacilli.

METHODS

Mycobacterium tuberculosis strains. Six strains of *M. tuberculosis* were used of various origins and characteristics (Table 1). All strains, except H37Ra and 79112R, had been freeze-dried or held at -20°C or in liquid nitrogen since their original isolation. During this study the strains were maintained on Löwenstein-Jensen medium without potato starch (Jensen & Kiaer, 1959). Organisms were taken directly from this medium for use in virulence tests. For all other experiments the bacteria were serially subcultured at least three times at 7 day intervals at 37°C in 25 ml Universal bottles which contained 10 ml of Middlebrook 7H9 liquid medium (Difco) enriched with bovine albumin fraction V (2.5 mg ml^{-1} ; Armour Pharmaceuticals, Eastbourne, Sussex) and glucose (1 mg ml^{-1}) but without catalase. This serial transfer in liquid medium ensured a grossly clump-free suspension of bacteria in the exponential phase of growth. The bacteria in the final subculture were collected by centrifuging at 1800 g for 15 min and resuspended in either water (for H_2O_2 susceptibility tests) or appropriate buffer. When necessary, suspensions were diluted after microscopical estimation of bacterial concentration (Thoma counting chamber).

Virulence tests. The root-index of virulence of each of the six strains of tubercle bacillus was evaluated at the end of the series of experiments as described by Mitchison *et al.* (1961). Five male Hartley strain guinea-pigs weighing 400 to 500 g were used for each bacterial strain and pathological changes were assessed 6 weeks after intramuscular inoculation.

Tests of the effect of pH and of interaction between pH and H_2O_2 on bacterial survival. A chequerboard experimental design (Cruickshank *et al.*, 1975) was used to test the effect of different concentrations of H_2O_2 on bacterial survival at different pH values in mixtures of 50 mM-citric acid and 50 mM-sodium phosphate. Preliminary experiments showed that this buffer was less bactericidal than either 50 mM-acetate or 50 mM-lactate buffers at pH 5.0. A stock solution of H_2O_2 (BDH) was standardized using the ϵ_{230} value of $81\text{ l mol}^{-1}\text{ cm}^{-1}$ and from this solution final concentrations of 0, 0.005, 0.02 and 0.08 % (w/v) H_2O_2 were prepared in buffer at each 0.5 pH unit from pH 4.0 to 7.5 inclusive. These conditions were selected to embrace 0.02 % H_2O_2 , a concentration which had proved useful in distinguishing between strains of tubercle bacilli with high and low degrees of virulence (Subbaiah, Mitchison & Selkon, 1960; Mitchison *et al.*, 1963), and pH values which might be expected to arise in the mycobacterial environment in infected animals (Sprick, 1956). Survival was assessed in a total of 32 experimental conditions for each of the six bacterial strains as follows. Duplicate 25 ml Universal bottles containing 3.8 ml of buffer were brought to 37°C in a water bath before adding 0.1 ml of an appropriate dilution of H_2O_2 and 0.1 ml of bacterial suspension in buffer to give a final concentration of about 1.0×10^6 bacteria ml^{-1} . Samples (0.1 ml) were removed after 45 and 90 min incubation at 37°C and serially diluted in water; the number of colony-forming units (c.f.u.) present was assessed on 7H11 agar (Cohn, Waggoner & McClatchy, 1968).

The dependence of survival of organisms on pH, H_2O_2 concentration and the two factors in combination was investigated by analysis of variance. Orthogonal linear contrasts (Davies, 1967) were employed to determine whether the relationship between pH and survival ($\log_{10}$ c.f.u. ml^{-1}) was linear or quadratic and whether the concentration of H_2O_2 influenced this relationship.

Superoxide susceptibility tests. The effect of $\cdot\text{O}_2^-$ on the survival of tubercle bacilli was examined at two pH values using a modification of the method of Babior, Curnutte & Kipnes (1975) in which $\cdot\text{O}_2^-$ was generated enzymically by the oxidation of purine with xanthine oxidase.

Tubercle bacilli were suspended at a concentration of about 4×10^5 organisms ml^{-1} in a reaction buffer which comprised 67 mM-glucose, 5 mM-purine, 500 μM -EDTA and either 40 mM-sodium acetate at final pH 5.4 or 25 mM-potassium phosphate at final pH 7.0. To duplicate $10 \times 75\text{ mm}$ glass tubes containing 500 μl of bacterial suspension were added 400 μl of 1.25 times strength buffer, 50 μl of water, or water containing 33 μg superoxide dismutase (SOD; Sigma) and/or 10 μg catalase (Boehringer). The tubes were brought to 37°C , 50 μl of xanthine oxidase (167 μg protein; Boehringer) or water (control) was added and, after 45 and 90 min of further incubation at 37°C , 100 μl samples were removed, diluted 10-fold in water and c.f.u. ml^{-1} was assessed on 7H11 agar.

The flux of $\cdot\text{O}_2^-$ generated in these systems was assessed using 1.0 μM -cytochrome *c* (Boehringer) as an

Table 1. Characteristics of *M. tuberculosis* strains

Strains showing a root-index of virulence in the guinea-pig of $>$ or < 1.0 were termed high- or low-virulence strains respectively (Mitchison *et al.*, 1963). Strains were defined as resistant (R) or susceptible (S) to 0.02 % H_2O_2 as described by Subbaiah *et al.* (1960) and to isoniazid on the basis of growth or no growth on Löwenstein-Jensen medium which contained 50 μ g isoniazid ml^{-1} .

Strain	Origin	Virulence	Catalase activity	H_2O_2 susceptibility	Isoniazid sensitivity
I2646*	British patient	High	+	R	S
79499*	Indian patient	High	+	R	S
H37Ra	NCTC7417	Low (group 3)	+	R	S
B1453†	British patient	Low (group 2)	—	S	R
79112*	Indian patient	Low (group 1)	+	S	S
79112R‡	Mutant of 79112	Low	—	S	R

* Data from Mitchison *et al.* (1963).

† Unpublished data of D. A. Mitchison & B. W. Allen.

‡ Derived from the parent strain 79112 as a mutant resistant to 50 μ g isoniazid ml^{-1} and characterized as described in the text.

electron acceptor for $\cdot O_2^-$. The reaction was started by the addition of xanthine oxidase and cytochrome *c* was either present at the outset or was added 2 h after the start of the reaction. The initial rate of change in extinction at 418 nm was measured and the flux was calculated using the ϵ_{418} value for cytochrome *c* (reduced minus oxidized) of 70000 $l\ mol^{-1}\ cm^{-1}$ (Salin & McCord, 1974). Immediately after addition, xanthine oxidase caused a $\cdot O_2^-$ flux of 3.9 μ mol $ml^{-1}\ min^{-1}$ at pH 5.4 and 9.4 μ mol $ml^{-1}\ min^{-1}$ at pH 7.0. SOD inhibited these rates by 82 and 88 % respectively. Two hours after the initiation of the reaction the fluxes had decreased by only 7 % at pH 5.4 and 12 % at pH 7.0.

Bacterial extracts. Bacteria from ten 10 ml cultures were pooled, centrifuged and resuspended in 1 ml of ice-cold 1.0 mM-potassium phosphate buffer, pH 7.0, then disrupted by ultrasonication in an ice bath (five 1 min periods with 1 min intervals) using a Biosonik (Bronwill Scientific Co., Rochester, New York, U.S.A.) microprobe at 60 W output. The extract was centrifuged at 14000g at 4 °C for 30 min and samples of the supernatant were either stored at -20 °C for up to 2 weeks before enzyme and protein assays or kept at 4 °C and assayed for catalase activity within 24 h.

Enzyme characterization by polyacrylamide electrophoresis. Bacterial extracts were diluted in 1.0 mM-phosphate buffer to a concentration of 1 mg protein ml^{-1} and 50 μ l samples were subjected to electrophoresis on 10 % or 7.5 % (w/v) polyacrylamide gels, 5 cm length, at pH 8.3 (Davis, 1964; Bohnenkamp & Weser, 1975). SOD was identified in the presence and absence of 1 mM-cyanide with the staining method of Salin & McCord (1974). Catalase and peroxidase activities were detected using diaminobenzidine with and without horse-radish peroxidase (Boehringer) respectively (Gregory & Fridovich, 1974). Peroxidase activity was also detected using *o*-dianisidine (Gayathri Devi *et al.*, 1975).

Enzyme assays. SOD activity was quantified in electrophoresis gels as described by Bohnenkamp & Weser (1975) using bovine SOD (Sigma) as a standard. Catalase (EC 1.11.1.6; hydrogen-peroxide:hydrogen-peroxide oxidoreductase) was assayed by the method of Peters, Müller & de Duve (1972) and peroxidase (EC 1.11.1.7; donor:hydrogen-peroxide oxidoreductase) was estimated using *o*-dianisidine substrate (Klebanoff, 1965). Protein was assayed by the Lowry method but using sodium citrate instead of sodium tartrate (Leggett Bailey, 1967).

Fig. 1. Effect of pH and of H_2O_2 on the survival of *M. tuberculosis* strains suspended in citrate/phosphate buffer at different pH values at 37°C . Survival was assessed after 45 and 90 min incubation with 0, 0.005, 0.02 and 0.08 % H_2O_2 . The results for strains B1453 and 79112R are those obtained at 45 min; with strain B1453 there was no survival using 0.02 or 0.08 % H_2O_2 and with strain 79112R there was no survival using 0.005 to 0.08 % H_2O_2 . For both strains, survival after 90 min exposure to H_2O_2 was not detected. The results for the other strains were obtained at 90 min. The effect of 0.005 % H_2O_2 was minimal with these strains and, for clarity, the data have been omitted; with strain 79112 there was no survival using 0.08 % H_2O_2 . The horizontal line represents 100 % survival. $\circ-\circ$, Buffer alone; $\bullet-\bullet$, 0.005 % H_2O_2 ; $\bullet---\bullet$, 0.02 % H_2O_2 ; $\bullet\cdots\bullet$, 0.08 % H_2O_2 .

RESULTS

The effects of pH and of H_2O_2 at different concentrations and pH values on survival of each of the strains of tubercle bacillus are shown in Fig. 1. With strains I2646, 79499, 79112 and H37Ra survival in the absence of H_2O_2 was poorest at low and high pH values after both 45 and 90 min incubation and optimum in the pH range 5.0 to 6.5. This quadratic effect of pH on the logarithm of the number of surviving organisms was highly significant ($P < 0.001$) in each instance. Strains B1453 and 79112R showed a linear decrease in survival with decrease in pH. No relationship was apparent between virulence and acid resistance.

H_2O_2 was bactericidal against all strains and, as expected, increasing the H_2O_2 con-

Fig. 2. Viability of *M. tuberculosis* strains after 90 min at 37 °C in the presence of xanthine oxidase-generated $\cdot\text{O}_2^-$ at pH 7.0 with and without the addition of SOD and catalase. $\square$, $\cdot\text{O}_2^-$ -generating system only; $\boxtimes$, $\cdot\text{O}_2^-$ -generating system+SOD; $\blacksquare$, $\cdot\text{O}_2^-$ -generating system+catalase; $\boxplus$, $\cdot\text{O}_2^-$ -generating system+SOD+catalase. Results were calculated as:

$$\frac{\text{c.f.u. ml}^{-1} \text{ in test conditions} - \text{c.f.u. ml}^{-1} \text{ in buffer control}}{\text{c.f.u. ml}^{-1} \text{ in buffer control}} \times 100.$$

centration resulted in greater kill. Strains I2646, 79499 and H37Ra resembled each other in showing a high degree of resistance to 0.08 % H_2O_2 . In contrast, strains B1453, 79112 and 79112R were all fully sensitive to 90 min exposure to 0.08 % H_2O_2 but differed markedly from each other in their sensitivities to lower concentrations and shorter exposure. Strain 79112R was the most sensitive and B1453 was more sensitive than 79112.

The bactericidal effect of H_2O_2 was influenced by H^+ concentration; pH values least favourable for bacterial survival permitted the greatest effect of H_2O_2 . Analysis of the 90 min exposure results revealed a significant increase in the effect of H_2O_2 at both high and low pH values for strains 79499, H37Ra and 79112 ($P < 0.001$, < 0.05 and < 0.001 respectively) and at low pH values for strain I2646 ($P < 0.001$).

Between-strain differences in susceptibility to the $\cdot\text{O}_2^-$ -generating system paralleled differences in sensitivity to H_2O_2 . In three independent experiments strains I2646, 79499 and H37Ra showed no loss of viability in the presence of $\cdot\text{O}_2^-$ when tested at pH 7.0 but killing occurred in at least two out of three experiments with strains B1453, 79112 and 79112R. Typical results are shown in Fig. 2. H_2O_2 arises spontaneously from $\cdot\text{O}_2^-$ by dismutation according to the reaction $2\cdot\text{O}_2^- + 2\text{H}^+ \rightarrow \text{O}_2 + \text{H}_2\text{O}_2$. Catalase consistently protected the strains which were susceptible to the $\cdot\text{O}_2^-$ -generating system but SOD, which catalyses the dismutation reaction, had no protective effect when added alone or together with catalase. Hence H_2O_2 was the cause of bacterial death and no $\cdot\text{O}_2^-$ -dependent kill could be discerned. At pH 5.4 the results were broadly similar to those seen at pH 7.0 but complicated by tendency of the bacterial suspensions to clump; survival was enhanced by catalase, but not SOD, with strains B1453 and 79112R (results not shown).

All six strains of tubercle bacillus had cyanide-resistant SOD activity. Only one SOD band was found by electrophoresis on either 7.5 % or 10 % polyacrylamide gels and this had a mobility which was the same for each strain and distinct from that of either of the SODs of *Escherichia coli* or bovine erythrocyte. Similar amounts of SOD activity were found in each of the strains (Table 2).

Catalase and peroxidase activities were absent from strains B1453 and 79112R. Both activities were found in the remaining strains (Table 2) and the activities could not be

Table 2. *Enzyme content and virulence of M. tuberculosis strains*

Enzyme activities are average values from duplicate or triplicate bacterial cultures and expressed as units (mg extracted protein)⁻¹. Replicate values were within 25 % of their respective means.

Strain	SOD	Catalase activity	Peroxidase activity	Root-index of virulence $\pm$ s.d.
I2646	1.16	7.9	320	1.17 $\pm$ 0.18
79499	0.92	10.0	400	1.29 $\pm$ 0.12
H37Ra	1.19	8.1	290	0.52 $\pm$ 0.22
B1453	0.89	NA	NA	0.77 $\pm$ 0.28
79112	0.81	7.7	370	0.92 $\pm$ 0.08
79112R	0.98	NA	NA	0.50 $\pm$ 0.09

NA, No activity detected.

separated by electrophoresis, thus supporting the conclusion of Gayathri Devi *et al.* (1975) that the activities are due to one enzyme. The electrophoretic mobility of the enzyme was the same for all four strains.

The results of assays of the virulence of the bacterial strains in the guinea-pig (Table 2) confirmed that strains I2646 and 79499 had high virulence (root-index > 1.0) and the other strains were of lower virulence, as expected on the basis of previous studies in this laboratory (Table 1). The virulence of strain 79112R was significantly lower than that of the parent strain 79112 ($P \approx 0.01$).

DISCUSSION

Earlier observations suggested that resistance to H_2O_2 was associated with virulence in tubercle bacilli. The loss of virulence which occurred on mutation to isoniazid resistance was accompanied by loss of resistance to H_2O_2 [for references see Mitchison *et al.* (1963)]. In addition, even among isoniazid-sensitive tubercle bacilli freshly isolated from patients, a significant correlation was found between virulence and resistance to H_2O_2 (Mitchison *et al.*, 1963; Narayanan Nair *et al.*, 1964). Association of H_2O_2 susceptibility with low virulence in native and isoniazid-resistant tubercle bacilli (low-virulence groups 1 and 2) was confirmed in this study but the results also showed that resistance to H_2O_2 cannot be the sole determinant of virulence in tubercle bacilli. Strain H37Ra (low-virulence group 3), although highly attenuated, was at least as resistant to H_2O_2 as the virulent strains I2646 and 79499.

The features of H37Ra which are responsible for its low virulence remain unknown. Hart & Armstrong (1974) showed that the viability of H37Ra declined in mouse peritoneal macrophages *in vitro* while the viability of the virulent parent strain H37Rv did not. With H37Ra, phagosomes were more often fused with lysosomes but it was not clear whether bacterial death preceded or followed exposure to lysosomal contents. Comparisons between H37Ra and H37Rv have also shown differences in amino acid metabolism (reviewed by Ramakrishnan, Suryanarayana Murthy & Gopinathan, 1972) but the relationship of such differences to virulence is unclear.

H_2O_2 susceptibility correlates with low catalase content in isoniazid-resistant tubercle bacilli (group 2) but not in native tubercle bacilli (group 1) (Mitchison *et al.*, 1963). Accordingly, H_2O_2 susceptibility and low virulence can be ascribed to catalase deficiency in isoniazid-resistant strain B1453 (group 2) but not in strain 79112 (group 1) which possesses as much catalase as the high-virulence strains I2646 and 79499. Possession of catalase is therefore only one of the bacterial features which confer resistance to H_2O_2 in tubercle bacilli but the nature of the other, missing in group 1 strains, is unknown. When 79112R was derived by mutation from 79112, loss of catalase was accompanied by further increase in H_2O_2 susceptibility, indicating that catalase and the unidentified bacterial

feature act independently in conferring H_2O_2 resistance. The importance of H_2O_2 resistance for virulence was further supported by the significant loss of virulence which also accompanied the mutation.

Correlations between virulence and H_2O_2 resistance and between virulence and lipid envelope composition have been independently demonstrated among the same group of isoniazid-sensitive tubercle bacilli isolated from patients (Mitchison *et al.*, 1963; Goren *et al.*, 1974*a, b*). However, it is not apparent how the differences in lipids could be causally related to differences in H_2O_2 resistance. Unsaturated lipids in the thick lipid envelope might protect the bacilli against H_2O_2 by offering a substrate for harmless peroxidation reactions and studies of the degree of unsaturation in the cell wall lipids would be of interest.

The present observations failed to support the hypothesis that low virulence might correlate with susceptibility to low pH. The optimum pH for survival and growth of tubercle bacilli *in vitro* is slightly acid (see for example Darzins, 1958) and the tubercle bacillus' environment in macrophages may be acidic, as suggested by the use of indicator dyes in casein-elicited mouse and guinea-pig peritoneal mononuclear phagocytes (Sprick, 1956) and by studies with pyrazinamide. [This drug is markedly tuberculocidal *in vitro* only at low pH (McDermott & Tompsett, 1954) but it is highly effective against tubercle bacilli in man (East African/British Medical Research Council Investigation, 1973), in mice (McCune *et al.*, 1966) and in rabbit macrophages infected *in vitro* (Mackanness, 1956).] However, no difference in optimum pH for survival was apparent between the virulent and less virulent strains tested here, using citrate/phosphate buffer which has low toxicity. Low pH values markedly increase the tuberculocidal effect of other organic acids, particularly under low oxygen tension (Dubos, 1953*a, b*). The possibility that virulence might correlate with resistance to lactic acid or other acids under such conditions (Dubos, 1953*b*) awaits further exploration.

Although bacilli of high and low virulence were comparably resistant to low pH, a possible role of low pH in host defence was indicated by the enhancement of H_2O_2 toxicity below about pH 5. The basis of this synergism is unknown. The activity of mycobacterial catalase is maximum at pH 7.5 (Diaz & Wayne, 1974) but the effect of pH on H_2O_2 susceptibility was the same in the catalase-negative and catalase-positive strains. Peroxide has least chemical reactivity around neutral pH (Hamilton, 1974).

At least part of the H_2O_2 generated by polymorphonuclear phagocytes is available in the microbial environment within these cells to take part in microbicidal reactions (Klebanoff, 1975) and H_2O_2 is probably simultaneously present with low pH. If the same is true of tubercle bacilli in macrophages, the combination of H_2O_2 and low pH would provide a tuberculocidal system of greater power than either H_2O_2 or low pH separately. H_2O_2 production accompanies, and is often enhanced by, phagocytosis in monocytes and macrophages from diverse host and tissue sources (reviewed by Klebanoff & Hamon, 1975). In endotoxin-elicited rat peritoneal macrophages, the pH in the vacuoles containing indicator-stained yeast declined to pH 3.5 to 4.0 within 10 to 15 min of phagocytosis (Jensen & Bainton, 1973). Hence, simultaneous presence of low pH and H_2O_2 within newly formed phagocytic vacuoles of macrophages seems possible. However, living tubercle bacilli, unlike yeast, can inhibit fusion of phagosomes with lysosomes (Hart & Armstrong, 1974) and it is not certain that pH declines in unfused phagosomes.

Resistance to $\cdot\text{O}_2^-$ is apparently not a virulence factor in tubercle bacilli. Macrophages from various sources have been shown to produce $\cdot\text{O}_2^-$ in addition to H_2O_2 (Drath & Karnovsky, 1975; Karnovsky *et al.*, 1975; Gee & Khandwala, 1976) and this free radical may be involved in microbicidal reactions in polymorphonuclear leukocytes (Johnston *et al.*, 1975; Weening, Wever & Roos, 1975) and monocytes (Sagone, King & Metz, 1976). However, even with strains H37Ra and 79112R, the least virulent of the tubercle bacilli tested, no $\cdot\text{O}_2^-$ -dependent killing of the bacteria was demonstrable *in vitro*. In addition, no correlation was apparent between tubercle bacillus SOD content and virulence. Mandell

(1975) has shown that virulence in *Staphylococcus aureus* also bears no relation to SOD content but correlates with catalase content.

Virulent tubercle bacilli evidently possess at least two independent means of resisting the lethal effects of H_2O_2 but loss of virulence can occur without loss of H_2O_2 resistance. The manner in which a host overcomes infection with H_2O_2 -resistant laboratory-attenuated tubercle bacilli remains unknown but it is clear that low virulence can be ascribed to different bacterial properties in native, isoniazid-resistant and laboratory-attenuated tubercle bacilli.

We thank Professor D. A. Mitchison for advice and assistance in the evaluation of guinea-pig pathology in virulence tests.

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