

## Relative Permissiveness of Macrophages from Black and White People for Virulent Tubercle Bacilli

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**Epidemiological, clinical, and histopathological evidence suggests that black people are more susceptible to tuberculosis than are white people. The cellular basis of this putative susceptibility was investigated in vitro by comparing responses of blood-derived macrophages from black and white donors to experimental infection with virulent tubercle bacilli. Phagocytes from pairs of black and white donors were infected. The uptake and replication of the tubercle bacilli in these cells were measured by microscopic counts and by CFU counts of bacilli at 0, 4, and 7 days. The effects of donor serum, of 1,25-(OH)<sub>2</sub>-vitamin D<sub>3</sub>, and gamma interferon on the infection also were studied. Black-donor phagocytes killed more bacilli during phagocytosis than white-donor phagocytes did. However, the bacilli grew consistently and significantly faster in successfully infected macrophages from black than from white donors, especially in the presence of black-donor serum. 1,25-(OH)<sub>2</sub>-vitamin D<sub>3</sub> gave significantly less protection against tubercle bacilli to macrophages from black donors than to macrophages from white donors. The permissiveness of the macrophages from the two races was affected equally by gamma interferon. These results demonstrate some inherent and environmental liabilities in the monocytic phagocytes and serum of black people compared with white people, which may contribute to their greater susceptibility to tuberculosis.**

Strains and races of experimental animals are differently susceptible to tuberculosis (1). Such differences might also exist among races of people. Tuberculosis is said to occur more frequently and be more difficult to treat in black people than in white people (2, 14, 20, 21). Its incidence was reported to be much higher among residents of Asiatic origin in the United Kingdom than among white residents (7).

The causes of such differences are probably both genetic and environmental. For instance, a gene for expressing resistance to tuberculosis has been identified in mice (1), and, environmentally, protein deprivation increases the susceptibility of guinea pigs to tuberculosis (12). The two causes are interconnected. This is illustrated by an explanation (7) for why the United Kingdom residents of Asiatic origin are more susceptible than the white residents. In the explanation, vitamin D is assumed to support antituberculosis resistance (14). Food in the United Kingdom is not supplemented with vitamin D. Consequently, the country's residents rely mainly on sunlight for this vitamin. The white residents, because of lighter skin, can photosynthesize vitamin D better than the dark-skinned residents of Asiatic origin (23, 24). They therefore should be able to maintain stronger antituberculosis resistance. In this explanation, lower photosynthesis of vitamin D is a genetic factor, and inadequate sunlight and lack of oral supplementation are environmental factors which interact to make the residents of Asiatic origin more susceptible to tuberculosis than the white residents.

Such interpretations, however, are inconclusive because of the many factors which cannot be known or controlled in epidemiological studies. A more definitive understanding of differences in response to tuberculosis infection between human individuals and races should be obtainable from fully controlled in vitro experiments with human macrophages and lymphocytes (3-5, 16). Such experiments have been able to demonstrate significant differences between individual

white subjects in cultured-macrophage susceptibility to tubercle bacilli (3, 5). In the present work they were done to compare monocytes and macrophages from white and black subjects for relative susceptibility to virulent tubercle bacilli and to look for cellular and molecular explanations of the reputedly greater susceptibility to tuberculosis of black than white people.

### MATERIALS AND METHODS

**Media and ingredients.** Human peripheral blood monocytes were obtained by centrifugation on Ficoll-Hypaque and were washed and plated as described fully elsewhere (3, 6, 8). In short, the leukocyte layer from 50 ml of arm vein blood centrifuged on Ficoll-Hypaque was collected. These cells were washed four times in Hanks solution and suspended at 10<sup>7</sup> nucleated cells per ml in RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.). The plating medium was supplemented with 2 mM L-glutamine, 0.5 mM sodium thioglycolate, and 1% unheated human serum (either autologous or AB, as indicated below). AB serum for these experiments was obtained from a normal white male donor who has provided neutral control serum for experiments done in this laboratory for several years (3). Sodium thioglycolate was omitted from the medium used to culture the cells after plating.

1,25-(OH)<sub>2</sub>-vitamin D<sub>3</sub> (1,25-D<sub>3</sub>) was a gift of Milan R. Uskokovic, Hoffman-La Roche Inc., Nutley, N.J. It was kept as a stock solution of 1 mg in 1.5 ml of 95% ethanol, stored at -20°C, and diluted into the culture medium for use, as previously described (6). Recombinant human gamma interferon was a gift of Genentech, Inc., South San Francisco, Calif., and was prepared, diluted, and used as previously described (9). The bacteriologic culture media 7H9 broth and 7H10 agar were purchased from Difco Laboratories, Detroit, Mich., or BBL Microbiology Systems, Cockeysville, Md. Other chemicals were reagent grade.

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**Methods of culturing and infecting the monocytic phagocytes.** Full descriptions of culture and infection methods have been published elsewhere (3, 6, 8). Briefly, the procedure was as follows. The freshly isolated suspension of monocytes was distributed to 35-mm bacteriologic-grade petri dishes (Falcon no. 1008; Becton Dickinson Labware, Oxnard, Calif.) at three 50- $\mu$ l droplets per dish. After incubation for 30 min at 37°C in 7.5% CO<sub>2</sub> in air, the nonadherent cells were washed off and discarded. When the adherent cells were to be tested as monocytes, they were infected immediately after the nonadherent cells had been washed off. When they were to be tested as macrophages, they were incubated for 7 days before infection. The properties of these monocytic phagocytes in this experimental model of tubercle bacillus infection have been fully described previously (3).

These cells were incubated in 1.5-ml volumes of medium. For infection, they were exposed for 30 min, except in experiments on the effects of time on infection, to medium usually containing  $7.2 \times 10^5$  tubercle bacilli per ml. In some experiments, higher and lower concentrations of bacilli were used, as will be noted. After infection, they were washed to remove bacteria. Medium was added back to the cells, and they were incubated for a further 7 days. Samples were taken at 0, 4, and 7 days after infection. Some samples were fixed and stained for phagocyte and acid-fast bacillus counts. Most samples were lysed, diluted, and plated to make bacterial CFU counts (3, 6, 8). The counts shown in the figures are CFU per milliliter of monocytic phagocyte lysate, the product of an average of  $1 \times 10^5$  macrophages or  $2 \times 10^5$  monocytes. Data are shown as means for samples or for experiments, with standard error bars and numbers of samples. Student's *t* test was used for statistical analyses. The procedures used for making the macrophage lysates with sodium dodecyl sulfate and diluting them for plating and CFU counting on 7H10 agar have been described previously (8). The bacteria used to infect the cells were the *Mycobacterium tuberculosis* Erdman strain prepared as described earlier (4).

**Human subjects.** Normal subjects for use as blood donors were recruited from among staff and students at this institution (informed consent was obtained). They ranged in age from the late 20s to late 40s. There were two female and four male black donors and five female and three male white donors. They were used in pairs, that is, the cells from a black donor and a white donor both were drawn and tested simultaneously with the same reagents and conditions.

## RESULTS

**Comparative rates of tubercle bacillus growth in macrophages from black and white donors.** Experiments comparing the uptake and growth of tubercle bacilli in macrophages from black and white donors were done as illustrated by the model experiment whose results are shown in Fig. 1. Macrophages from a pair of donors were infected simultaneously and under the same conditions. Samples of the infected macrophages were taken at 0, 4, and 7 days, and CFU were counted. CFU counts were plotted against time after infection. Data for initial uptake, for rates of bacterial replication (mean generation time, *G*, in hours), and for 7-day CFU were analyzed.

Data from the experiment in Fig. 1 show two important findings of this research. One is that tubercle bacilli multiply faster in macrophages from black than from white donors (lower *G*) and reach higher concentrations by the end of the incubation (7-day CFU). The other is that zero time CFU

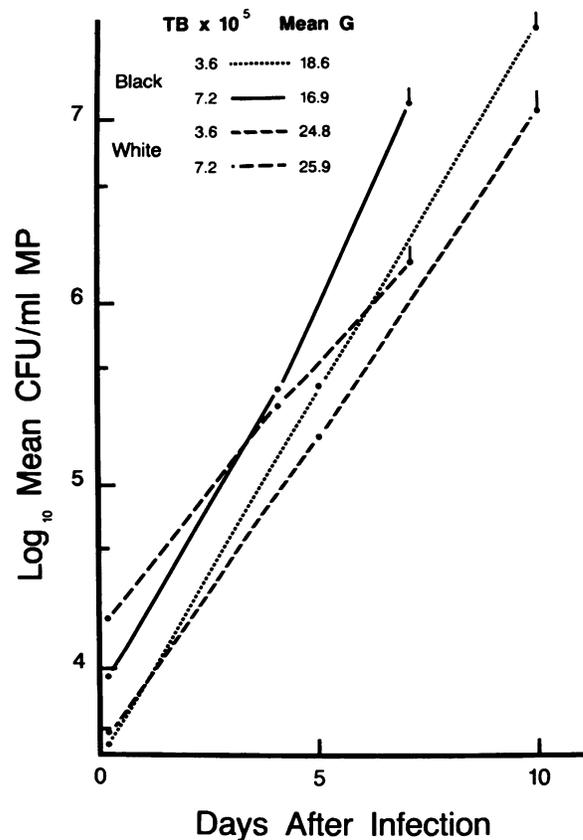


FIG. 1. Results from a single model experiment illustrating the kinds of data that are summarized for multiple experiments in other figures. CFU are plotted against time in days after infection for black- and white-donor macrophages infected with  $3.6 \times 10^5$  or  $7.2 \times 10^5$  tubercle bacilli (TB) per ml. CFU were counted at 0, 5, and 10 days for the lighter infection and at 0, 4, and 7 days for the heavier one. Each datum point is a mean of five values, and bars denoting standard error of the mean are shown for 7- and 10-day CFU. Growth rates of the intracellular bacilli are shown as mean generation times (*G*) in hours.

usually are lower in macrophages from black than from white donors. In most experiments, only  $7.2 \times 10^5$  tubercle bacilli per ml and a 7-day incubation period were used. However, when other conditions were used, for instance infection with  $3.6 \times 10^5$  bacilli per ml and a 10-day incubation (Fig. 1), the same results were obtained.

Eighteen experiments like the one just described were done to compare tubercle bacillus growth in macrophages from black and white donors. Fourteen of the experiments used paired donors. The donors were six blacks (two females and four males) and eight whites (five females and three males). Mean *G* values for all of these experiments are shown in Fig. 2. These data show that tubercle bacilli multiply significantly faster in black-donor macrophages in autologous serum than white-donor macrophages in autologous serum ( $P < 0.001$ ). They suggest a similar difference for the respective macrophages in AB serum, but the difference was not statistically significant ( $P < 0.05$ ). The results indicate that macrophages from black donors are more permissive than those from white donors and that incubating the macrophages in autologous serum magnifies this difference.

**Relative ability of 1,25-D<sub>3</sub> to protect macrophages from**

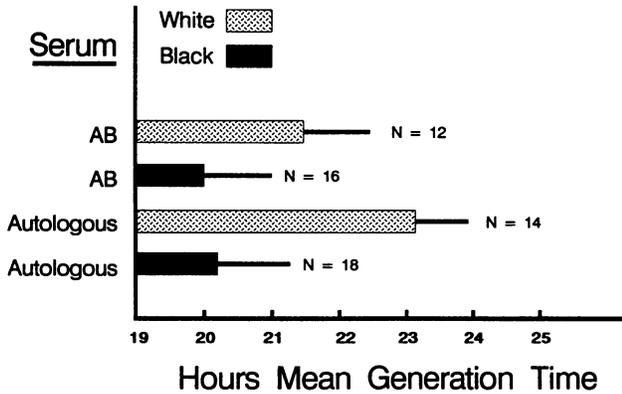


FIG. 2. Mean generation times of tubercle bacilli in black- and white-donor macrophages incubated in autologous or AB serum. Data are from 18 experiments, 14 with pairs of donors. Differences between means for black- and white-donor cells in autologous serum are significant at  $P < 0.001$ . Each bar is shown with a line denoting standard error of the mean, and the number of samples (N) is given.

**black and white donors against tubercle bacilli.** Of the 18 experiments done with macrophages from black and white donors, 10 included groups comparing the protectiveness of  $4 \mu\text{g}$  of 1,25- $\text{D}_3$  per ml for the macrophages in a regimen known to protect white-donor macrophages consistently (6). The results (Fig. 3) show that 1,25- $\text{D}_3$  protects the macrophages of both races significantly ( $P < 0.001$ ) but also that it protects macrophages from white donors better than macrophages from black donors in both autologous serum and AB serum ( $P < 0.001$  for both kinds of sera).

**Effects of gamma interferon on tubercle bacillus replication in macrophages from white and black donors.** Of the 18

experiments comparing macrophages from black and white donors, 9 included groups receiving 50 U of gamma interferon per ml to determine whether macrophages from black donors might be more permissive in the presence of gamma interferon (9, 15-17) than macrophages from white donors. The results are summarized in Fig. 4. They confirm earlier findings that gamma interferon does not protect human macrophages against tubercle bacilli. Macrophages from both races were somewhat more permissive in the presence of gamma interferon than in its absence. However, for this group of data and concentration of the interferon, the greater permissiveness was not statistically significant. No difference was evident between macrophages of the two races in expression of this tendency.

**Differences between black- and white-donor monocytic phagocytes in viable tubercle bacilli at time of infection.** In experiments to measure the differences between black- and white-donor monocytic phagocytes in tubercle bacilli, there were consistently fewer CFU in cells from black than from white donors at the zero-time sampling immediately following 30 min of infection. Microscopically, infected phagocytes from black donors had fewer acid-fast bacilli and more non-acid-fast bacteria than infected phagocytes from white donors. The lower CFU count is documented in Fig. 5 with data for macrophages from 13 different donors in 14 separate experiments, in which cells from paired donors were infected for 30 min under similar conditions. There were significantly ( $P < 0.001$ ) fewer culturable bacilli in black- than white-donor macrophages incubated in autologous serum. The same tendency was seen for cells incubated in AB serum, but the difference was not statistically significant.

**Effects of time and concentration of bacilli on CFU counts immediately after infection.** The evidence above suggested that some tubercle bacilli were being killed by phagocytes

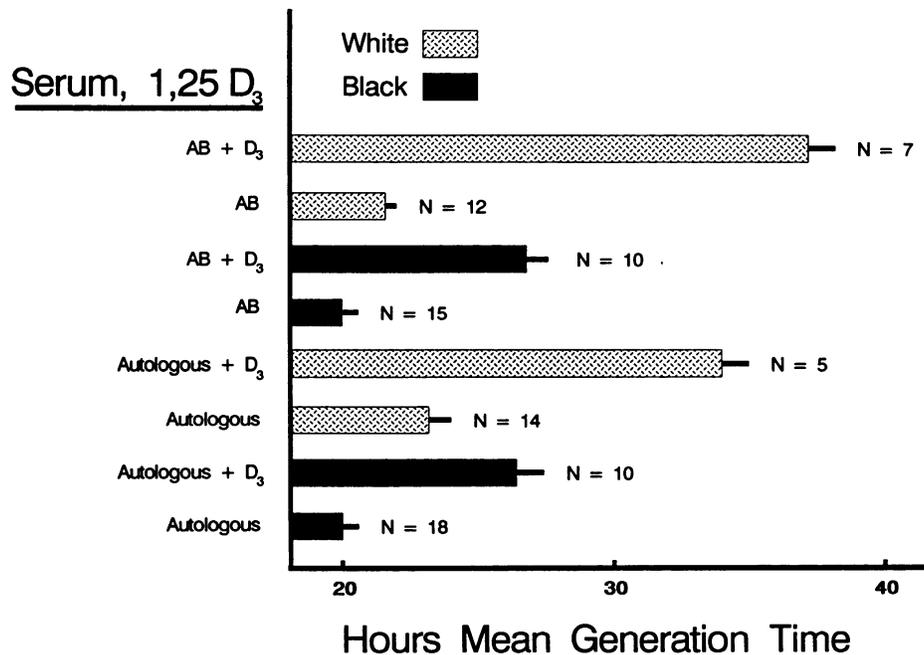


FIG. 3. Comparative anti-tubercle bacillus protection of black- and white-donor macrophages in AB or autologous serum by  $4 \mu\text{g}$  of 1,25- $\text{D}_3$  per ml. Results are from 10 experiments, 7 with paired donors. 1,25- $\text{D}_3$  was significantly ( $P < 0.001$ ) protective for macrophages from both black and white donors, but it was also significantly more protective ( $P < 0.001$ ) for macrophages from white than from black donors.

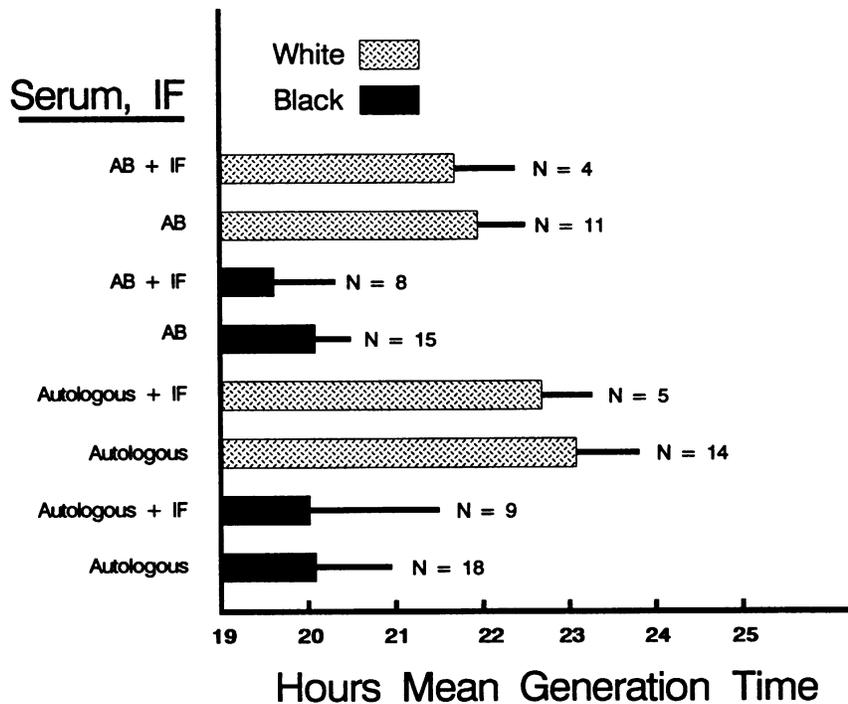


FIG. 4. Comparative effects of 50 U of gamma interferon (IF) per ml on permissiveness of macrophages from black and white donors for tubercle bacilli. Results are from nine experiments, five with paired donors. There was no significant difference between macrophages from black and white donors in increased permissiveness as a result of the presence of interferon.

from black donors during the 30-min period of infection normally used in these experiments. To examine this observation further, we performed five experiments with macrophages from paired donors in which 15-, 30-, and 60-min periods of infection were used. The cells were infected with four different concentrations of tubercle bacilli, over an eightfold range. In addition, four experiments were done with monocytes, that is, adherent monocytic phagocytes infected immediately after isolation from donor blood.

The results of these experiments are summarized in Fig. 6 and 7.

These data show the following. Uptake of CFU by monocytes and by macrophages was approximately equivalent (Fig. 6). This conclusion takes into account the fact that there were twice as many phagocytes in the monocyte cultures as there were in the macrophage cultures (see Materials and Methods). In both kinds of cell, CFU counts were closely proportional to the concentration of tubercle bacilli used for infection (Fig. 7). In monocytes from both black and white donors, the 30- and 60-min counts were below theoretical counts projected from 15-min values (Fig. 6) and CFU for the cells from the black donors were below those for the cells from the white donors. Counts were also lower at 30 and 60 min in macrophages from black than from white donors. Counts for both kinds of macrophage were much closer to projected theoretical values than were counts for monocytes. These data suggest that monocytes from black and white donors kill some tubercle bacilli during infection and that macrophages from white donors lose this activity.

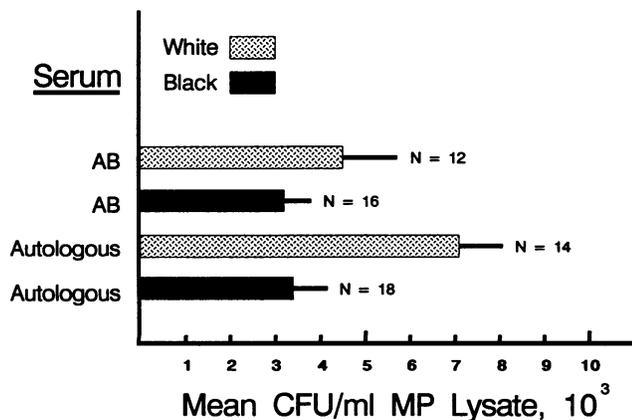


FIG. 5. Mean CFU in black- and white-donor macrophages infected for 30 min with  $7.2 \times 10^5$  tubercle bacilli per ml in medium supplemented with 1% AB or autologous serum. Data are from 18 experiments, 14 with paired donors. The difference between means for black- and white-donor macrophages in autologous sera was significant at  $P < 0.001$ .

DISCUSSION

Strain or race differences in resistance to tuberculosis exist in guinea pigs (19, 25), rabbits (11), and mice (1). They may also exist in races of humans. Blacks, for example, are reported to be more susceptible to tuberculosis than whites (2, 11, 20-22). Epidemiological, clinical, and histopathological evidence for this difference has been summarized by Rich (13). More recently, Davies (7) found that dark-skinned residents of Asiatic origin in the United Kingdom had 30 times more pulmonary tuberculosis than comparable white

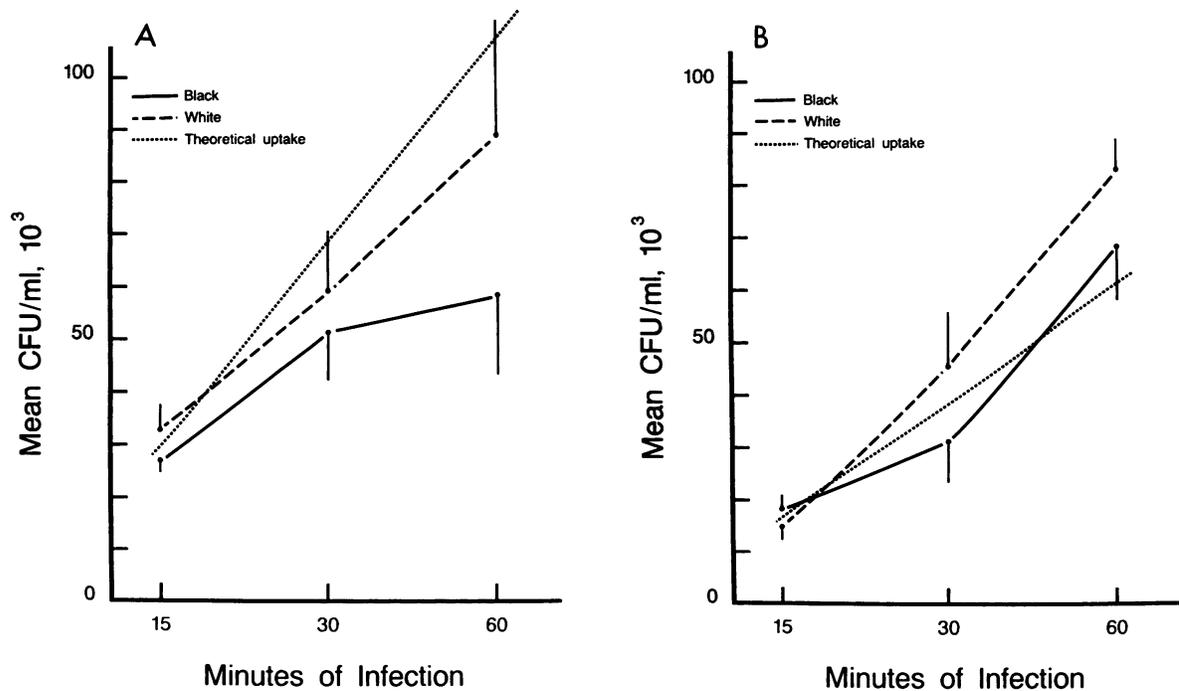


FIG. 6. Data from four experiments for monocytes (A) and five for macrophages (B), from paired black and white donors, showing mean CFU in black- and white-donor cells for 15-, 30-, or 60-min periods of infection with  $28 \times 10^5$  tubercle bacilli per ml. Theoretical slopes for CFU values projected from 15-min CFU counts if no bacilli were being killed during 30- and 60-min periods of infection are also shown. Similar results were obtained with 3.6, 7.2, and 14 bacilli per ml (see Fig. 7).

residents. He suggested that this was because of both genetic and environmental factors relating to the protectiveness of vitamin D against tuberculosis (14). The genetic factor was dark skin and consequent reduced vitamin D photosynthesis (23, 24), and the interacting environmental factors were inadequate sunlight and insufficient intake of vitamin D (7).

Epidemiological evidence for differences among human races in antituberculosis resistance is inconclusive because of its inherent lack of adequate controls. Better evidence is provided by the controlled experiments with cultured macrophages described here. Tested under the same conditions, macrophages from black donors tended to be more permissive for tubercle bacilli than those from white donors. This greater permissiveness was accentuated by the presence of autologous serum in the culture medium: black-donor serum increased the permissiveness, whereas white-donor serum decreased it. Macrophages from black donors also were not as well protected as those from white donors by  $1,25\text{-D}_3$ , a hormonally active form of vitamin D.

The greater permissiveness of macrophages from black donors and their lower responses to protective hormone would appear to be genetic predispositions to tuberculosis for black people. If vitamin D is important in antituberculosis resistance (14), the reduced ability of black people to photosynthesize the vitamin because of dark skin pigmentation (23, 24) would be an additional inherited liability. Nutrition and exposure to sunlight would be interdependent environmental factors. Environmental factors might have been responsible for differences in macrophage permissiveness related to the donor serum used in the culture medium.

In these experiments, CFU counts directly after infection

were lower for macrophages from black donors than for macrophages from white donors. Detailed investigation of this observation by using both monocytes (adherent cells infected just after isolation) and macrophages (adherent cells cultured for 7 days before infection) indicated that the lower counts probably were caused by greater killing of tubercle bacilli by phagocytes from black donors than by those from white donors during infection. The most direct evidence for this rapid type of killing, described previously for human macrophages (3), was bacterial disintegration seen microscopically in the phagocytes. Additional evidence was provided by differences between actual CFU counts at 30 and 60 min of infection and counts that could be projected for 30 and 60 min of infection from those at 15 min of infection. Monocytes from both black and white donors showed this killing ability, but it was greater in cells from black donors than in cells from white donors. It was diminished in macrophages from black donors and was weak or absent in macrophages from white donors. Since the number of peroxisomes decreases in human monocytic phagocytes as they mature from monocytes to macrophages (18), this rapid type of bacillary killing may be due to the peroxidase-peroxide-chloride bactericidal system of phagocytic monocytes as suggested by Lowrie (10). Its practical importance in human resistance to tuberculosis remains to be determined. It might make monocytic phagocytes from blacks more resistant than those of whites to initial infection with tubercle bacilli. However, the rate of development of infection, once established, should depend largely on the relative permissiveness of successfully infected cells, and this permissiveness was greater in cells from black donors than in cells from white donors.

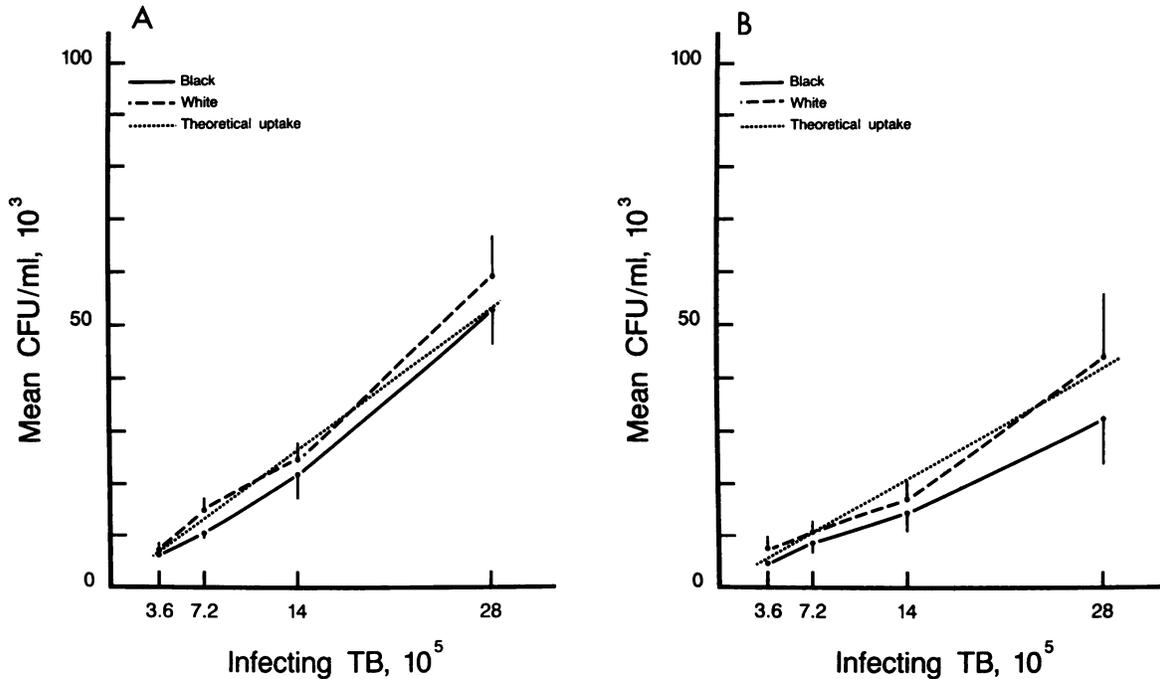


FIG. 7. Data from the experiments described in legend to Fig. 6, graphed to show the relationship between concentration of tubercle bacilli (TB) in the infecting suspension and CFU for monocytes (A) and macrophages (B) infected for 30 min. Similar data were obtained for 15- and 60-min infections (data not shown; see Fig. 6).

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