5. V. V. Khorobrykh, A. V. Pronin, A. F. Kirkin, et al., Immunologiya, No. 3, 76 (1983).
6. I. G. Tsyrlova, K. V. Gaidul', and V. A. Kozlov, Functional Characteristics of the Circulatory Pool of Immunocompetent Cells [in Russian], Novosibirsk (1984), p. 53.
7. I. G. Tsyrlova and V. V. Cheglyakova, Cellular Factor in the Regulation of Immunogenesis [in Russian], Novosibirsk (1985), p. 115.
8. V. V. Cheglyakova, I. G. Tsyrlova, S. V. Sennikov, et al., Abstracts of Proceedings of an All-Union Scientific Conference [in Russian], Angarsk (1986), pp. 271-272.
9. T. C. Cheng, S. K. Formar, and H. J. Kasesian, J. Biol. Chem., 249, 1781 (1974).
10. D. Coliavo, G. Biasi, A. Colombatti, et al., Br. J. Cancer, 32, 331 (1975).
11. A. J. Cunningham, Nature, 201, 1106 (1965).
12. K. A. Dicke, G. Tridente, and D. W. van Bekkum, Transplantation, 8, 422 (1969).

FUNCTIONAL CHARACTERISTICS OF THE ANTIOXIDATIVE SYSTEM OF MYCOBACTERIA GROWN ON MEDIA MODIFIED BY PERFLUORODECALIN

N. I. Popkova, A. A. Yushchenko, V. A. Yurkiv, and O. A. Irtuganova

UDC 579.873.21:579.222:577.152.1]083.13:547.221

KEY WORDS: superoxide dismutase, glutathione transferase, glutathione peroxidase, mycobacteria, perfluorodecalin.

One trend in the improvement of culturing of mycobacteria is the production of artificial nutrient media corresponding most fully to the physiological needs of various representatives of the genus *Mycobacterium*. One factor leading to optimization of biomass accumulation by strains of mycobacteria during periodic culture is additional aeration of the nutrient medium with the aid of gas-carrying perfluorocarbon liquids, such as perfluorodecalin (PFD), whose oxygen capacity is over 40 vols. %. PFD was used for the first time as a support medium for growth of tissue culture cells [1] and during the development of methods of culture of *Mycobacterium leprae* [4].

An essential condition for the existence of oxygen-metabolizing microorganisms is integrity of their antioxidative protection system [8, 15]. This accounts for the interest in the study of the response of the antioxidative enzyme system of the medically important strains of mycobacteria *M. bovis* BCG and *M. lufu* to culture in liquid medium with the addition of oxygenated PFD.

EXPERIMENTAL METHOD

Cultures (12-14-day) of *M. bovis* BCG and *M. lufu* were obtained on Shkol'nikova's medium with PFD (see the paper by A. A. Yushchenko et al. in this issue*). Oxygen was passed through the PFD beforehand for 20 min. A culture of mycobacteria grown on Shkol'nikova's medium without PFD served as the control.

The mycobacteria were sedimented from the nutrient medium by centrifugation (in the experimental version the PFD was first removed) at 5000 g for 15 min and the supernatant was discarded; the residue was washed with 1 ml of K-phosphate buffer, pH 7.4, and recentrifuged under the same conditions. The resulting residue was resuspended in 1.5 ml of the above-mentioned buffer and frozen in liquid nitrogen and thawed 4 times. The disintegrated mycobacteria were sedimented at 5000 g (15 min) and the supernatant was drawn off and kept for 2 weeks at −40°C. Superoxide dismutase (SOD) activity was determined by the method in [5] at 30°C, glutathione transferase (GT) activity was determined relative to 1-Cl-2,4-dinitro-

*As in Russian original; the paper is not in this issue — Publisher.

Leprosy Research Institute, Ministry of Health of the USSR, Astrakhan'. (Presented by Academician of the Academy of Medical Sciences of the USSR V. I. Pokrovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 2, pp. 187-189, February, 1988. Original article submitted March 20, 1987.

TABLE 1. Activity of Enzymes of the Glutathione Redox System and SOD in *M. bovis* BCG Grown on Medium with PFD

Enzyme	Shkol'nikova's medium		p
	without PFD (control)	with PFD (experimental)	
SOD, units/mg protein	383,7±40,0 (10)	392,45±40,92 (10)	>0,05
GT	52,85±4,2 (10)	36,33±4,0 (10)	<0,05
GP-TBH (mmoles/mg protein)	11,36±0,85 (8)	10,84±1,6 (10)	>0,05
GP-H_2O_2 (mmoles/mg protein)	1200,45±98,8 (9)	597,6±126,3 (10)	<0,05

TABLE 2. Activity of Enzymes of Antioxidative System of *M. lufu* Grown on Medium with PFD

Enzyme	Shkol'nikova's medium		Significance of differences between experimental and control values, p
	without PFD (control)	with PFD (experimental	
SOD, units/mg protein	249,9±27,7 (9)	432,6±56,4 (9)	<0,05
GT	65,4±4,67 (9)	70,5±3,9 (9)	>0,05
GP-TBH (mmoles/mg protein)	27,5±2,81 (10)	20,14±2,0 (9)	>0,05
GP-H_2O_2 (mmoles/mg protein)	1910,8±210,0 (10)	1509,0±95,9 (9)	>0,05

benzene at 37°C [6], and glutathione peroxidase (GP) activity was determined relative to hydrogen peroxide (GP-H_2O_2) and to tert-butyl hydroperoxide (GP-TBH) at 30°C [11]. Gultathione reductase activity also was measured at 30°C as described in [3], but activity of this enzyme was not discovered in test samples of all series. The protein concentration in the experimental material was determined by Lowry's method. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Cultivation of *M. bovis* BCG on medium modified with PFD was shown to be accompanied by a fall of GP activity (by 45.57%) and of GP-H_2O_2 activity (by 100.88%). SOD and organic GP (GP-TBH) activity was preserved at the control level (Table 1). The decrease in power of the antitoxic system of *M. bovis* BCG in response to an increased oxygen concentration in the nutrient medium may be evidence of the lability of the system studied and its transition in this particular situation to a new physiological level of control. However, reduction of activity of the hydrogen peroxide detoxicating enzyme (GP-H_2O_2) against the background of unchanged activity of the most general cytosol H_2O_2 generator (SOD), is grounds for a possible imbalance in the functioning of the antitoxic system under extremal conditions.

In the case of *M. lufu*, grown on medium with PFD, the character of changes in activity of the enzymes of their antioxidative defense was opposite in direction (Table 2). For instance, SOD activity increased by 42.23% compared with the control, evidently as a result of activation of apoenzymes or systems of their biosynthesis. The level of the remaining enzymes tested did not differ from that in mycobacteria grown on Shkol'nikova's medium without PFD. An increase in SOD activity against the background of the control GP-H_2O_2 level may be a factor involved in the increase in reserve power of the antitoxic system of *M. lufu*, grown on medium with PFD. In turn, the absence of significant changes in the activity of enzymes utilizing organic and inorganic peroxides and hydroperoxides (GT, GP-TBH, GP-H_2O_2) suggests that enrichment of the culture system with oxygen does not induce a burst of peroxidation processes, and that, consequently, it is physiological.

The results of these investigations indicate that addition of PFD to the culture system gives rise to antibathic changes in activity of the enzymes of antioxidative defense in different representatives of the genus *Mycobacterium*. Significant differences between the attenuated strain *M. bovis* BCG and the saprophytic, unclassified strain *M. lufu*, isolated from river mud in Zaire [12], with respect to the control levels of activity of the enzymes studied (Fig. 1), also determine the different response of the antitoxic systems of the two species of mycobacteria to the addition of supplementary oxygen to the culture medium.

On the whole, culture of mycobacteria on liquid medium modified with PFD was favorable for both groups. It led to definite changes in function of the enzymes of the glutathione

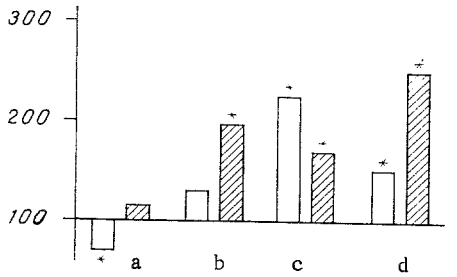

Fig. 1. Activity of enzymes of antioxidative system of *M. lufu* relative to *M. bovis* BCG, grown on media without PFD (unshaded columns) and with PFD (shaded columns). Data given in per cent of control, taken as activity for *M. bovis*. a) SOD; b) GT; C) GP-TBH; d) GP-H_2O_2. Asterisk indicates significance of differences between values compared at the $p < 0.05$ level.

redox system and SOD, optimal for each member of the genus *Mycobacterium*. In the case of *M. bovis* BCG, intensification of peroxidation could take place during culture by this method. This hypothesis is supported by the increase in GT and GP-H_2O_2 activity (Table 1).

The increase in SOD activity, against the background of constant levels of GP-TBH, GT, and GP-H_2O_2 levels in *M. lufu*, grown on medium with PFD, must be taken into account when optimal conditions are sought for growth of the agent of leprosy. We know that *M. leprae* cells die under the influence of myeloperoxidase and hydrogen peroxide [7, 13, 14], and that the cause of imperfect phagocytosis in leprosy may be an effect of "quenching" of free-radical processes [2], due to increased activity of SOD of bacterial origin and, consequently, a deficiency of superoxide anion-radicals and hydroxyl radicals in the macrophages [9, 10]. It can therefore be claimed that growth of *M. leprae* requires conditions ensuring increased SOD activity and inhibition of peroxidation processes. In this connection the addition of PFD to the culture system may be one factor which satisfies these requirements adequately.

LITERATURE CITED

1. F. F. Beloyartsev, Perfluorcarbons in Biology and Medicine [in Russian], Pushchino (1980), pp. 5-21.
2. L. I. Gasanova, Current Problems in Leprology [in Russian], Astrakhan' (1984), pp. 80-83.
3. A. M. Gerasimov, L. A. Koroleva, O. S. Brusov, et al., Vopr. Med. Khimii, 22, No. 1, 89 (1976).
4. A. A. Yushchenko and O. A. Irtuganova, Current Problems in Leprology [in Russian], Astrakhan' (1984), pp. 21-25.
5. C. Beauchamp and I. Fridovich, Anal. Biochem., 44, 276 (1976).
6. W. H. Habig, M. J. Pabst, and W. B. Jacoby, J. Biol. Chem., 249, 7130 (1974).
7. S. J. Klebanoff and C. C. Shepard, Infect. Immun., 44, 534 (1984).
8. E. Kusunose, M. Kusunose, and S. Isumi, FEMS Microbiol. Lett., 10, 49 (1981).
9. Y. Niwa and M. Ozaki, J. Clin. Hematol. Oncol., 24, 1039 (1983).
10. Y. Niwa, T. Sakane, Y. Miyachi, et al., J. Clin. Microbiol., 20, 837 (1984).
11. D. E. Paglia and W. N. Valentine, J. Lab. Clin. Med., 70, 158 (1967).
12. F. Portaels, Ann. Soc. Belg. Med. Trop., 60, 381 (1980).
13. A. K. Sharp and D. K. Banerjee, Acta Leprol. (Geneva), 2, 259 (1984).
14. A. K. Sharp, M. J. Colston, and D. K. Banerjee, J. Med. Microbiol., 19, 77 (1985).
15. P. R. Wheeler and D. Gregory, J. Gen. Microbiol., 121, 457 (1980).