COURSE OF EXPERIMENTAL TUBERCULOSIS IN ANIMALS PRELIMINARILY ACCLIMATIZED TO HYPOXIA

V. M. Davydova

Laboratory of Experimental Pathology and Therapy (Head, G. S. Kan),
Leningrad Research Institute of Tuberculosis (Director, Professor
A. D. Semenov)
(Presented by Academician V. N. Chernigovskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 56, No. 10,
pp. 35-40, October, 1963
Original article submitted November 15, 1962

The study of physiological mechanisms of immunity to turberculosis has necessitated the elucidation of the relationships between the specific and nonspecific factors in the phenomena of natural and postvaccinal resistance to Mycobacterium tuberculosis. This article reflects one stage of this study. It contains details of the course of experimental tuberculosis in animals preliminarily acclimatized to a lowered barometric pressure leading to the develop of hypoxia.

Several workers have found that during acclimatization to hypoxia compensatory reactions develop in the animal body and the general tissue resistance is raised, a fact of benefit in counteracting not only the hypoxia, but also a wide range of "extraordinary" stimuli: cyanides, ethyl alcohol, caffeine [2], strychnine [8], hypothermia, anemia [6], various infections [1, 7-10], and so on.

EXPERIMENTAL METHOD

Two series of experiments were conducted on albino mice weighing 19-22 g. In the first series of experiments 34 mice were acclimatized to hypoxia in a pressure chamber at an "altitude" of 4500 m for 4 h daily for 1 month as described by Barbashova [4]. In the second series of experiments 41 mice were acclimatized in the pressure chamber at an altitude of 7600 m by the same method. In each series an equal number of animals received no treatment whatever and acted as controls.

TABLE 1. Macroscopic Changes in the Lungs of Mice of the Control and Experimental Groups in the First Series of Experiments

Group of animals	Day after inoculation																																							
	5-th						15-th						18-th						28-th						50-th						78-th											
	–	+	++	+++	++++		–	+	++	+++	++++		–	+	++	+++	++++		–	+	++	+++	++++		–	+	++	+++	++++		–	+	++	+++	++++						
Control	3	1	—	—	—		—	—	3	1	—		—	—	—	1	3		—	—	—	—	4		—	—	—	2	2		—	—	—	—	4						
Experiment	4	—	—	—	—		—	3	1	—	—		—	—	3	1	—		—	—	—	4	—		—	—	—	4	—		—	—	—	—	4						

Legend: – no visible changes; + solitary punctate grey tubercles; ++ a few punctate grey tubercles; +++ a few submiliary and many punctate tubercles; ++++ many disseminated submiliary tubercles and a few confluent.

TABLE 2. Progress of Histological Changes and Presence of M. tuberculosis in Organs of Mice of the Experimental and Control Groups

Organ	Group of animals	Day after inoculation: 5-th								15-th								18-th							
		−	+	++	+++	++++	A	B	C	−	+	++	+++	++++	A	B	C	−	+	++	+++	++++	A	B	C
Lungs	Control		1	3				4		—	—	—	4	—	—	4	—				2	2		2	2
	Experimental		1	3			1	3		—	1	3	—	—	—	4	—			1	3			4	
Liver	Control		2	2			1	3				4				4					4			3	1
	Experimental		4				1	3			2	2			1	3			4					3	
Spleen	Control	2	2					4		1	1	2	2			4			1		4			3	1
	Experimental	4					2	2				2				4					3			4	

Continued

Organ	Group of animals	Day after inoculation: 28-th								50-th								78-th						
		−	+	++	+++	++++	A	B	C	−	+	++	+++	++++	A	B	C	−	+	++	+++	++++	A	B
Lungs	Control					4		4						4		4						4	1	3
	Experimental				1	3	1	3					2	2	2	2						4	3	1
Liver	Control		3	1				4			4				3	1			4				4	
	Experimental		4				2	2		1	3				3	1			4				4	
Spleen	Control			4				4			4				4				4				4	
	Experimental			4				4			4				2	2		3	1				4	

Legend: − no visible changes; + solitary foci in macrophages and lymphoid cells; ++ a few scattered foci; +++ many scattered foci; ++++ foci mainly confluent. M. tuberculosis: A) not found; B) present in small numbers; C) present in large numbers.

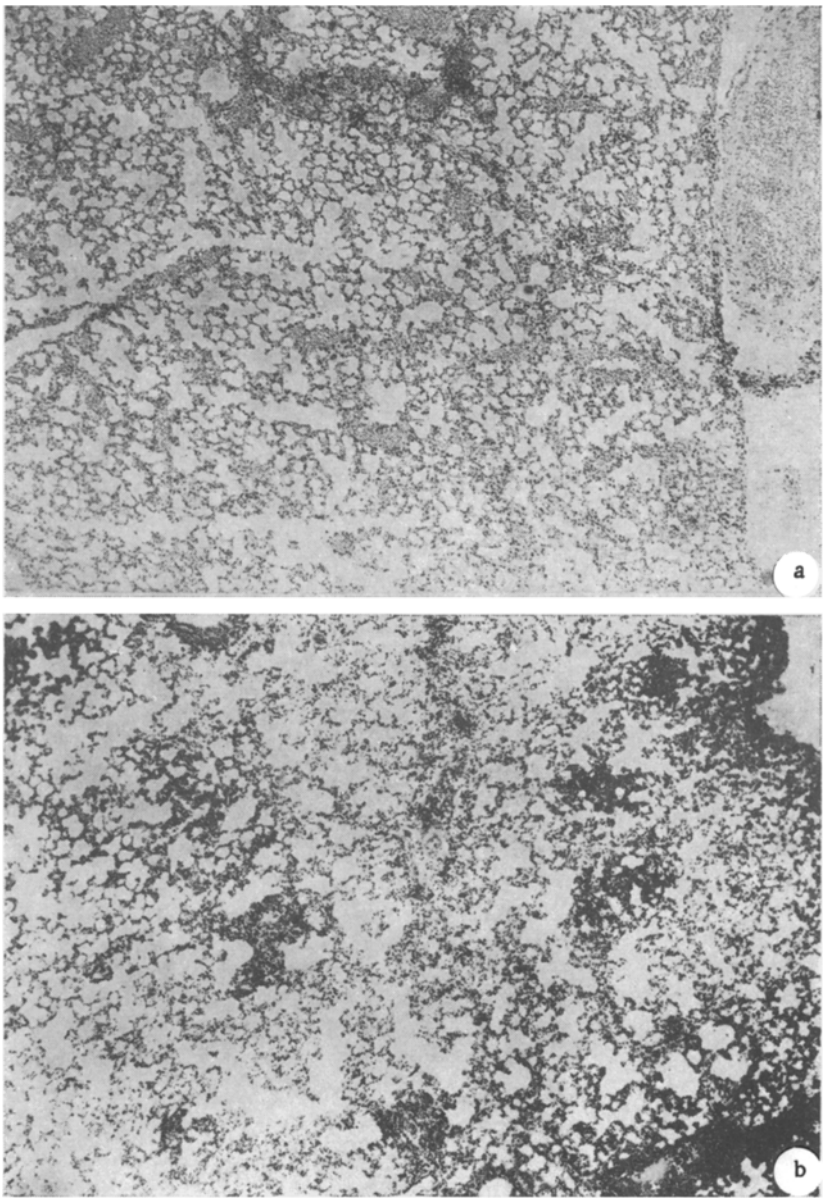

Fig. 1. Specific changes in the lungs of an experimental (a) and a control (b) mouse on the 15th day after inoculation. a) Solitary collections of lymphoid cells in the vessels; b) numerous disseminated foci of macrophages and lymphoid cells. Low power.

At the end of the period of acclimatization the tolerance of the animals to acute hypoxia at an "altitude" of 11,000 m was tested. The mean time from the beginning of the stay at a high altitude to the beginning of the "descent" (the "descent" was made when convulsions began to develop) in the acclimatized mice of the first series of experiments was 3.5 times greater, and in the acclimatized mice of the second series 7 times greater, than in the control animals.

At the end of the period of acclimatization all the mice of the experimental group together with the control mice were inoculated with M. tuberculosis (bovine type, strain No. 109) in a dose of 0.05 mg in 0.25 ml physiological saline by intravenous injection. At various intervals after inoculation (in the first series of experiments on the 5th, 15th, 18th, 28th, 50th, and 78th days and in the second series on the 6th, 15th, 22nd, 36th, 58th, and 82nd day) the animals were autopsied (4 from each group). The relative weight of the spleen and lungs (ratio between the weight of the organ and the body weight of the mouse) was determined and the internal organs (lungs, liver, and spleen) were investigated macroscopically and histologically.

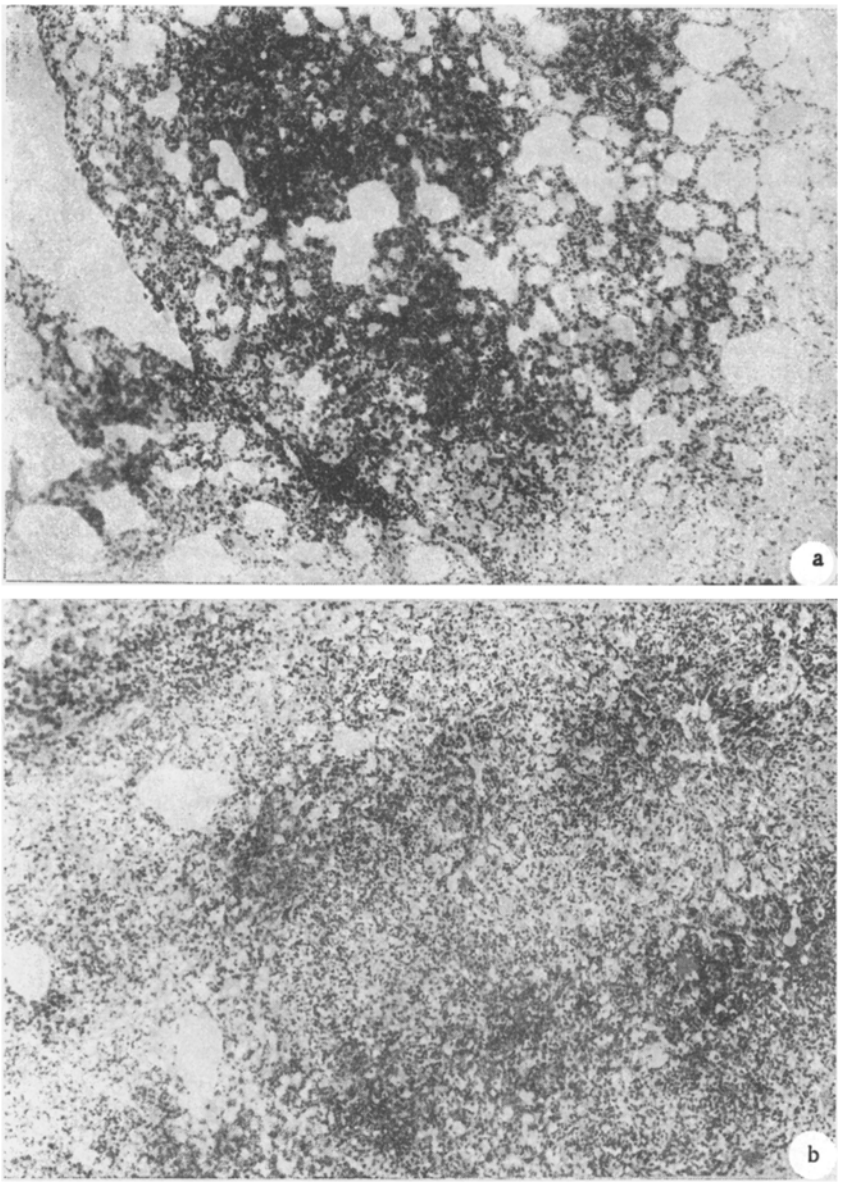

Fig. 2. Specific changes in the lungs of an experimental (a) and a control (b) mouse on the 36th day after inoculation. a) Numerous disseminated foci of macrophages and lymphoid cells; b) mainly confluent foci of macrophages and lymphoid cells with perifocal edema. Low power.

EXPERIMENTAL RESULTS

It may be concluded from the results given in Table 1 that the specific changes in the lungs of the experimental group of mice were rather less marked than those in the lungs of the control mice. This was most conspicuous on the 15th and 18th days after inoculation and less so at the subsequent periods of observation.

No changes were found as a result of macroscopic examination of the liver and spleen. In the control group enlargement of the spleen was observed from the 5th day after inoculation, on the 28th day a slight reduction in its size, and on the 50th day it was again enlarged. Enlargement of the spleen was also found in the experimental group, but it developed rather later than in the control animals – from the 15th day after inoculation. The subsequent stages of the process were correspondingly postponed: a loss in weight was noted on the 50th day and enlargement on the 78th day after inoculation.

The degree of severity of the specific histological changes in the organs of the experimental group of mice was less than that of the control animals on the 15th, 18th, and 50th days after inoculation (Table 2). On the 15th day after inoculation numerous disseminated foci of macrophages and lymphoid cells were found in the lungs of the mice of the control group, whereas in the lungs of the experimental mice only solitary, very small collections of lymphoid cells were found in 3 cases (Fig. 1), and in one case no changes whatever were found. Further progression of the specific changes in the lungs of the experimental mice took place more slowly than in the controls; confluent foci appeared only at the end of the period of observation, and they were somewhat smaller in size.

In the liver differences appeared on the 15th, 28th, and 50th days after inoculation; in the control group the lymphoid-epitheloid nodules were more numerous and larger in size than in the experimental group of mice.

In the spleen of the experimental group of mice a reduction in the severity of the changes was also observed on the 5th, 15th, 18th, and 78th days after inoculation by comparison with the controls. On the 5th day after inoculation no changes were found in the experimental mice, but in the controls solitary small nodules of epithelioid cells were seen in two cases. On the 15th day a few disseminated epitheloid nodules were found in 2 experimental mice, solitary nodules in one mouse, and no changes in one mouse; in the control group epitheloid nodules were numerous in 2 mice and a few, scattered nodules were present in another 2 mice. On the 78th day no changes were found in 3 experimental mice and in one there were solitary, small epitheloid nodules; many disseminated epitheloid nodules were found in all the control mice.

Mycobacteria tuberculosis were fewer in number on bacteriological examination of sections of the organs of the experimental animals than of the controls.

Hence, the results of the first series of experiments demonstrate that preliminary acclimatization to hypoxia slightly reduces the severity and delays the course of experimental tuberculosis in albino mice. In the animals of this series of experiments the course of the tuberculosis was relatively mild, and none of the animals died before the end of the period of observation.

In the second series of experiments the course of the tuberculosis in the mice was more intensive and the degree of acclimatization to hypoxia was more marked than in the animals of the first series of experiments. Specific changes were found on the 6th day of observation; at subsequent periods rapid progression of the lesion was found, and individual control animals died from the 31st day after inoculation and experimental animals from the 57th day. The mean survival period of the control mice was 53 ± 3.4 days, and of the experimental mice 81 ± 3.3 days.

The specific changes in the lungs and spleen of the mice of the experimental group were less severe than those in the control animals at all periods of observation starting from the 15th day. For instance, on the 36th day after inoculation (Fig. 2) numerous disseminated foci of macrophages and lymphoid cells were observed in the lungs of the mice of the experimental group, while in the lungs of the control animals the foci were of the same character but mainly confluent, in some places with perifocal edema. Mycobacteria tuberculosis were also fewer in the sections of the organs of the experimental mice than in those of the controls; they were found in the lungs of the experimental mice during examination with the immersion objective of the microscope, and in the lungs of the control mice with the low and high power. No differences were found in the liver.

Hence, in the experimental conditions described above, preliminary acclimatization to hypoxia increases the resistance of albino mice to subsequent inoculation with a virulent strain of M. tuberculosis. The increased resistance was manifested by a decrease in the severity of the specific morphological changes in the animals sacrificed at different periods after inoculation and also by a smaller number of Mycobacteria tuberculosis in the organs of the preliminarily acclimatized animals than in those of the control mice. The mean survival period of the preliminarily acclimatized mice was 53% longer than that of the control animals. The increased resistance was less marked in the animals with the more severe tuberculosis (second series of experiments) although the degree of acclimatization was higher in the animals of this series than in those of the first series.

The results of these experiments confirm the views of some authors [5, 11, 12, 13] that nonspecific factors are concerned, along with specific factors, in immunity to tuberculosis. It should be pointed out that the intensity of the nonspecific immunity attained in these experiments was lower than in the experiments by workers who increased the inborn immunity of albino mice by BCG vaccination (other conditions being equal).

SUMMARY

The work was done to ascertain the role played by nonspecific factors in the phenomena of natural resistance to Mycobacteria tuberculosis. Data are presented characterizing the course of experimental tuberculosis in albino mice, preliminarily acclimatized to hypoxia. The results of observations demonstrated that in the case of the model described preliminary acclimatization to hypoxia increased the resistance of albino mice to the subsequent infection with virulent Mycobacteria tuberculosis.

LITERATURE CITED

1. N. V. Balanina, Arkh. pat. 9, 4, 86 (1947).
2. Z. I. Barbashova and A. G. Ginetsinskii, Transactions of the I. P. Pavlov Physiological Institute of the AN SSSR [in Russian], Vol. 1, p. 103. Moscow-Leningrad (1945).
3. Z. I. Barbashova, Doklady Akad. Nauk SSSR 101, 2, 379 (1955).
4. Z. I. Barbashova, Acclimatization to Hypoxia and its Physiological Mechanisms [in Russian]. Moscow-Leningrad (1960).
5. G. S. Kan, in book: Study of the Role of the Nervous System in the Pathogenesis, Immunogenesis, and Treatment of Tuberculosis [in Russian], No. 2, p. 243, Leningrad (1961).
6. I. R. Petrov, Hypoxia of the Brain [in Russian]. Leningrad (1949).
7. V. V. Turanov, in book: Hypoxia [in Russian], p. 135. Kiev (1949).
8. L. J. Berry, R. B. Mitchell, and D. Rubinstein, Proc. Soc. exp. Biol. (N. Y.), Vol. 88, p. 543 (1955).
9. A. Loew, Physiologie des Höhenklimas. Berlin (1932).
10. S. G. Ong, Ann. Inst. Pasteur, Vol. 76, p. 415 (1949).
11. S. Raffel, Bull. Un. int. Tuberc., Vol. 30, p. 57 (1960).
12. A. Saenz, Sem. Hop. Paris. Path. et Biol., Vol. 9, p. 1463 (1961).
13. E. Suter, Experientia (Basel). Vol. 16, 258 (1960).

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
