

IMPORTANCE OF THE DEGREE OF AERATION OF THE MEDIUM FOR THE ANTIBACTERIAL ACTION OF DRUGS USED IN TUBERCULOSIS

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The development of *Mycobacterium tuberculosis* is dependent on the presence of oxygen in the medium [4]. In the macroorganism, the mycobacteria are often found in foci of necrosis (sometimes inside macrophages), where the oxygen concentration is lower than in the atmosphere, and this may affect their vital activity. The bactericidal action of streptomycin and isoniazid on the strain *M. tuberculosis* var. *humanum* H 37 Rv in anaerobic conditions has been found to be weakened [5]. No definite information is available regarding other antituberculosis drugs, although it may be assumed that the action of kanamycin (of the neomycin group) on mycobacteria must be dependent on the oxygen concentration, for such a relationship has been established for the effect of other antibiotics of this group on members of the orders of the Coccaceae and Eubacteriales [1, 2].

In the present communication the results are given of an investigation of the importance of aeration of the medium to the action of the following chemotherapeutic preparations on growth of *M. tuberculosis*: tubazid (isoniazid), D-cycloserine, D,L-cycloserine, thianide (ethionamide, treacator), kanamycin, and diambutol (ethambutol).

METHODS

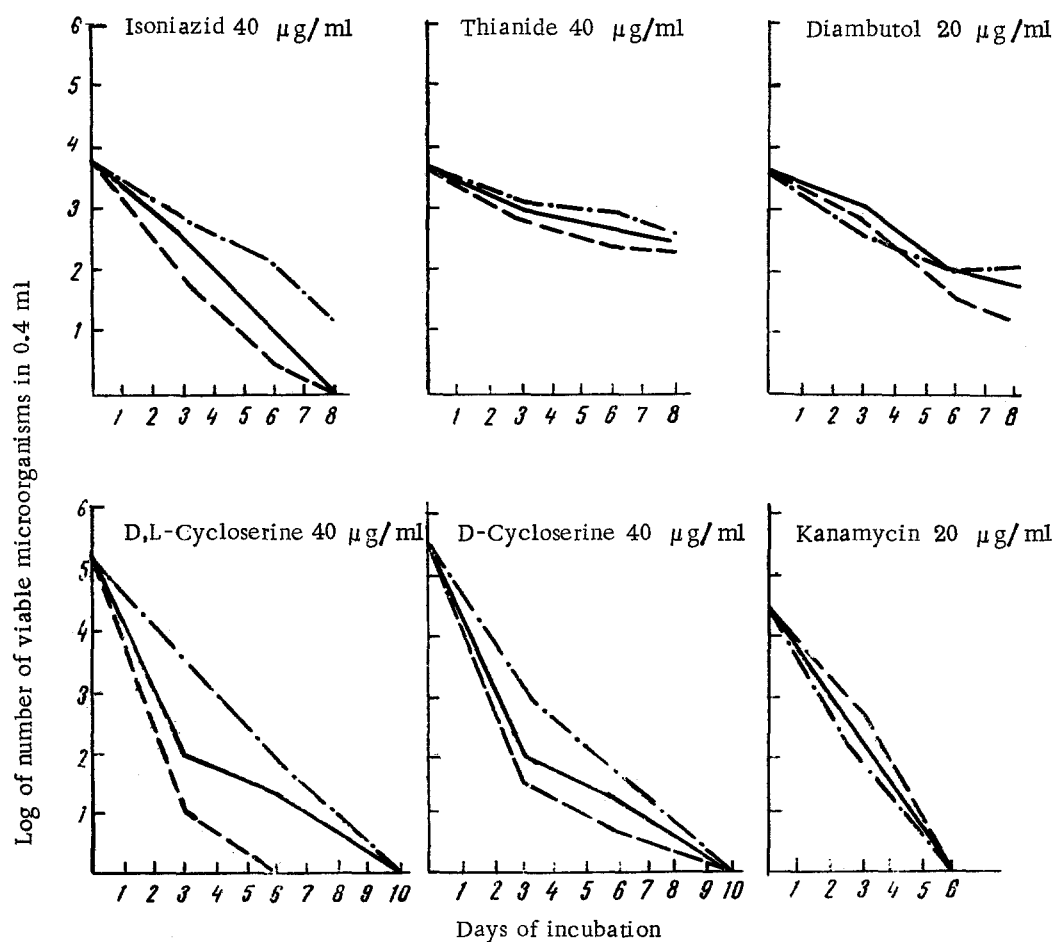
A strain of *M. tuberculosis* var. *avium* was used, which was isolated from a patient at the Central Institute of Tuberculosis, Ministry of Health of the USSR and kindly presented by B. Ya. Stukalova.

A 10-12 day culture of the mycobacteria on Petragnani's medium was used in the experiments. The action of the chemotherapeutic substances was studied in Soton's medium with serum. In the first variant of the experiments the bactericidal action of the preparations was studied in a liquid medium. Incubation took place at 37° in ordinary aerobic conditions with a lowered oxygen concentration (in an anaerostat at a pressure of 1 atm) and with intensive aeration (agitation about 100 times per minute). The change in the oxygen concentration in these conditions was shown by the rate of proliferation of the microorganisms (studied in additional experiments)—highest in the presence of agitation, lower in standard aerobic conditions and very low in the anaerostat. To determine the number of viable microorganisms, seedings were taken from the flasks every 2-3 days on to Petri dishes with 1.5% Soton's agar medium with serum.

The bacteriostatic action of the preparations was studied in columns with semiliquid medium and on the surface of solid medium. Tubes with Soton's medium, containing 0.3% agar-agar, were heated in a boiling water bath to remove air, and then cooled to 50°, at which temperature serum was added to the medium together with the suspension of microorganisms and various dilutions of the preparations to be tested. In parallel experiments the same suspension of mycobacteria was applied to the surface of a 1.5% agar medium. The tubes were incubated at 37° and the growth of the colonies was read every 7 days.

Inhibition of Growth of *M. Tuberculosis* Var. *Avium* Under Influence
of Antituberculosis Preparations in Media with Different Levels of Aeration

Preparation	Medium	Bacteriostatic concentrations of preparations (in $\mu\text{g/ml}$) at periods of observation men- tioned below		
		7 Days	14 Days	21 Days
Tubazid (isoniazid)	Solid	10	40	100
	Semiliquid	10	200	200
Thianide (ethionamide)	Solid	2	10	40
	Semiliquid	10	40	100
Kanamycin	Solid	1	4	10
	Semiliquid	2	10	10
D-Cycloserine	Solid	10	20	20
	Semiliquid	10	20	20
D,L-Cycloserine	Solid	4	10	10
	Semiliquid	10	10	20
Diambutol	Solid	10	10	20
	Semiliquid	20	20	20



Importance of aeration of medium for bactericidal action of antibacterial preparations on *M. tuberculosis* var. *avium* 430. — incubation in standard aerobic conditions; --- incubation in semi-anaerobic conditions; - - incubation with agitation of flasks containing medium.

RESULTS

The details given in the table show that the differences in the value of the bacteriostatic concentration of the preparation in relation to M. tuberculosis (when tested on the surface of solid medium and in agar columns) were most clearly seen in the experiments with isoniazid and thianide. These differences were due to the fact that with high concentrations of the preparations, growth of the colonies was inhibited in the upper part of the column, but was present in the lower part, which was less saturated with oxygen. The degree of aeration of the medium was less important in the experiments with kanamycin and D,L-cycloserine, and was completely without effect on the bacteriostatic action of D-cycloserine.

The study of the bactericidal action of the preparations showed that aeration influenced the rate of the effect produced by isoniazid and both cycloserine preparations. The rate of the bactericidal effect of diambutol, thianide, and kanamycin on the cells of M. tuberculosis was completely, or almost completely, independent of the oxygen saturation of the medium (see figure).

Hence, the degree of aeration had an effect on the action of all the antituberculosis drugs tested, although in the case of some preparations it was mainly the velocity of the bactericidal action that was changed, while in the case of others it was the size of the bacteriostatic concentration. For preparations with relatively weak bactericidal properties (thianide and diambutol) a very slight dependence of the velocity of the bactericidal action on the degree of aeration of the medium was established. However, no causative link was present evidently between these phenomena, for the marked bactericidal action of kanamycin was independent of the degree of aeration of the medium.

The cause of the conflicting results obtained during the investigation of the bactericidal and bacteriostatic action of thianide and cycloserine likewise remained unexplained. This discrepancy between the size of the bacteriostatic concentration of the preparation and the velocity of its bactericidal action with different degrees of oxygen saturation of the medium was also observed in our previous experiments with tetracycline [3], where it was explained by the possibility of the existence of two independent mechanisms of action of the antibiotic in bacteriostatic and bactericidal concentrations. Further investigations will make it possible to judge how far this explanation may also be extended to the results of the experiments with thianide and cycloserine.

SUMMARY

The degree of the inhibiting concentration of isoniazid, kanamycin ethionamide, ethambutol and D,L-cycloserine with regard to M. tuberculosis var. avium increases with a decrease in the oxygen content of the medium. The inhibiting concentration of D-cycloserine does not depend on the oxygen content of the medium. The bactericidal effect of isoniazid on tuberculosis mycobacteria grows with an increase in the aeration of the medium and slows down with its decline. An analogous relationship in a somewhat lesser degree was revealed in experiments with D,L- and D-cycloserine and was manifest slightly, if at all, in experiments with ethionamide, ethambutol and kanamycin.

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