RESPIRATORY CHARACTERISTICS OF *MYCOBACTERIUM LACTICOLA* O_{11}

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ABSTRACT

The effect of some substrates, respiratory inhibitors and streptomycin on the oxygen consumption of *Mycobacterium lacticola* O_{11} is studied using the Warburg manometric technique.

It is found that sodium lactate, sodium pyruvate and sodium acetate increase the oxygen consumption to a considerable extent, while sodium azide, potassium cyanide and streptomycin inhibit the oxygen consumption considerably.

Mycobacterium lacticola O_{11} is one of the eighteen micro-organisms isolated from the intestines of the Indian earthworm by Dr. Khambata and Dr. Bhat using the oxalate-enrichment technique. They carried out various cultural, morphological and nutritional characteristics and established the identity of the organism which was confirmed subsequently by Dr. Ruth E. Gordon of Rutgers University, U. S. A. (Khambata and Bhat, 1955 & 1957).

Our interest in this organism is due to its immunobiological properties in mice against tuberculosis. It has been established that the immunity produced by this organism is appreciable against infection induced by virulent strain of tubercle bacilli, the degree of immunity produced being equal to that conferred by B. C. G. under similar conditions (Gangadharam 1956, Gangadharam and Sirsi, 1959).

The results on the respiratory characteristics of *Mycobacterium lacticola* O_{11} reported here form a part of further series of experiments conducted to throw more light on the metabolism of this organism. They fall under two headings: (*a*) Action of various substrates on the oxygen consumption of *M. lacticola* O_{11}; (*b*) susceptibility of *M. lacticola* O_{11} to respiratory inhibitors and antibiotics.

METHODS

(i) *Culture*:—The bacterial mass was harvested by centrifuging at 4,000 r.p.m. for 20 minutes, a two-day old culture of the organism grown in nutrient

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broth contained in 50 ml. Erlenmeyer flasks. The cells were washed twice with distilled water and finally resuspended in M/15 phosphate buffer (pH 7.0) to give a dense homogeneous suspension. This was facilitated by triturating the mass of cells with a glass rod.

(ii) The respiratory studies mentioned here were carried out at 37.5°C using the conventional Warburg techniques. Each Warburg flask contained 1.0 ml. of the bacterial suspension and 1.25 ml. of the M/15 Sorensen's phosphate buffer (pH 7.0) in the main compartment and 0.25 ml. of the substrate or the inhibitor (or water) in the side-arm. The contents of the side-arm were tipped into the main compartment after 20 to 30 minutes when equilibrium was attained.

RESULTS AND DISCUSSION

Effect of substrates on the oxygen consumption of Mycobacterium lacticola O_{11}. The substrates tried were glycerol, glucose, sodium acetate, sodium succinate, sodium pyruvate, sodium citrate and sodium α-ketoglutarate at a final concentration of 0.02 M. The results expressed in the QN values are presented in Table I.

TABLE I

Effect of substrates on the oxygen consumption of *Mycobacterium lacticola* O_{11}

Substrate	Oxygen consumption expressed in QO_2 (N) values
Endogenous	1.70
Glycerol	9.48
Glucose	6.83
Sodium lactate	20.69
Sodium acetate	14.64
Sodium succinate	5.59
Sodium pyruvate	15.47
Sodium citrate	1.53
Sodium α-ketoglutrate	7.34

It is evident from the results that only sodium lactate, sodium acetate and sodium pyruvate could increase the oxygen consumption of *Mycobacterium lacticola* O_{11} to a considerable extent. On the other hand, glucose and glycerol gave only low values with this organism.

Effect of Respiratory inhtbitors on the oxygen consuption of Mycobaeterium lacticola O_{11} :—The effect of the respiratory inhibitors, potassium cyanide and sodium azide on the oxygen uptake of *Mycobacterium lacticola* O_{11} was studied at the final concentrations of 0.01 M and 0.001 M. The results are presented in Table II.

TABLE II

Inhibition of the respiration of *Mycobacterium lacticola* O_{11} by potassium cyanide and sodium azide

Inhibitor	Concentration	Percentage inhibition
Potassium cyanide	0.01 M	70.0
Potassium cyanide	0.001 M	45.6
Sodium azide	0.01 M	20.2
Sodium azide	0.001 M	18.9

The results presented above show that potassium cyanide possessed greater inhibitory properties on the oxygen consumption of *Mycobacterium lacticola* O_{11} than sodium azide. But it is to be noted that even with the high concentration of cyanide used in this study, the inhibition was not high enough to warrant us to call the organism cyanide sensitive. These studies were carried out using whole cell preparations and cell-permeability factor might play a part. However, it is difficult to definitely say, whether this organism possesses a cyanide sensitive or in-sensitive respiratory mechanism.

Effect of streptomycin on the oxygen consumption of Mycobacterium lacti cola O_{11} :—In our earlier studies it was observed that among several antibiotics and chemotherapeuticals tested, only streptomycin was found to be inhibitory to the growth of the organism at a concentration of 0.1 ug/ml. (Gangadharam, *1956*). The effect of streptomycin on the respiration of *Mycobaterium lacticala* O_{11} was therefore studied. The results are graphically represented in Fig. I.

It is evident from the graph that the antibiotic inhibited only about 30 % of the respiration of the organism. On the other hand, from our earlier studies, it was found to be highly inhibitory to the growth of the organism in nutrient broth. Perhaps factors like cell-permeability and other diffusion phenomena exert a greater influence on the action of the antibiotic on the respiration of the organism than its effect on the growth, probably due to the shorter time of investigation of the former effect. However, these findings show that streptomycin possesses some effect on the respiration of the organism besides its considerable activity on the growth. The importance of these findings comes to the fore-front when we consider the useful immunobiological properties of the organism against infections by virulent strains of tubercle bacilli. (Gangadharam 1956, Gangadharam and Sirsi, 1959.)

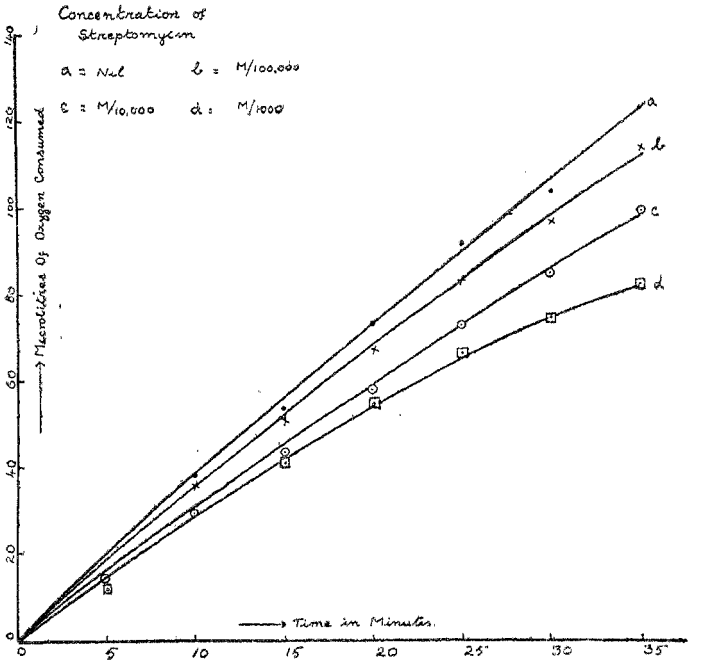

FIG. I

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REFERENCES

1. Gangadharam, P. R. J. — "Immunological and Chemotherapeutic studies in Tuberculosis." Membership thesis submitted to the Indian Institute of Science, Bangalore, (1956).
2. Gangadharam, P. R. J. and Sirsi, M. — *Indian J. Tuberc*, 1959, 7, 4
3. Khambata, S. R. and Bhat, J. V. — *J. Bact*, 1955, **69**, 227.
4. Khambata, S. R. and Bhat, J. V. — *Archiv. fur Microbiol*, 1957, **28**, 69