

THE EFFECT OF VARIOUS SUBSTANCES ON THE OXYGEN UPTAKE OF THE TUBERCLE BACILLUS

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Although there have been many studies on the factors affecting the growth of the tubercle bacillus the oxidative metabolism of these bacteria has received comparatively little attention. Loebel, Shorr, and Richardson (1933) used the Warburg technic and found that lactate, glycerol, salts of the higher fatty acids, glucose and lactose increased the oxygen consumption of the H37 strain. On the other hand, Nakamura (1938) found that carbohydrates, amino acids, ethyl alcohol, citric, formic, tartaric, succinic and malic acids had no effect on the oxygen consumption of human, bovine, or fowl strains. Glycerol had a very small accelerating action. In the experiments described below a bovine strain was used. Negative results were obtained with these substrates and also with pyruvic acids and certain amines. The salts of the higher fatty acids were found to cause a small acceleration. Other compounds have, however, a very marked effect.

EXPERIMENTAL

The bovine B₁ strain isolated at Saranac Lake was grown on beef and glycerol infusion broth. The cultures were used when they were from 2 to 6 weeks old and this variation in age had no effect on the results. The floating masses were removed with a loop and suspended in sterile saline in Hopkins tubes. They were then centrifuged at 2000 r.p.m. for 15 minutes. The saline was then replaced by 0.05 M phosphate buffer pH 6.7 so that

0.1–0.2 ml. of the packed bacteria was eventually suspended in 1.0 ml. of buffer. By rotating a glass rod the diameter of which was just smaller than that of the narrow part of the Hopkins tube the bacterial masses were broken up and an even suspension obtained. All the solutions were sterile but the Warburg vessels were not. The experiments were not run for more than 7 hours. Cultures taken at the end of this time showed an occasional small growth of *Pseudomonas aeruginosa*. When this organism was used under the same conditions as the tubercle bacillus only a few of the substances that affected the O₂ uptake of the latter had any effect on its uptake. This was also true for *Staphylococcus aureus*, *Escherichia coli*, and blastomycetes. The fact that the oxygen uptake was affected immediately on addition of the compounds is also good evidence that the tubercle bacillus was the only one involved.

Usually 0.5 ml. of the suspension of tubercle bacilli was used in each Warburg vessel. The substances to be tested were dissolved in phosphate buffer at pH 6.7 and the final volume made up to 2.0 cc. with buffer. Most of the effects recorded had their optimum between pH 6.5 and 7.0. The O₂ uptake and CO₂ production were measured in the usual way in air at 37°.

Aldehydes. All the aldehydes used increased the O₂ uptake of the tubercle bacilli. These include citral, heptaldehyde, isovaleraldehyde, anisaldehyde, benzaldehyde and furfural. The last two were the most effective and in certain concentrations doubled the O₂ uptake and CO₂ production. The question arises whether these compounds and the others described below are acting as stimulants to the normal metabolism of the bacteria or are being oxidized as substrates. Concentration curves for benzaldehyde and furfural are shown in figure 1. Within a certain range the extra O₂ uptake is proportional to the concentration of the two aldehydes but definite end points were not obtained. Table 1 shows that as the number of bacteria is increased the oxygen taken up for a given amount of aldehyde increases but this is not proportional to the bacterial concentration. o-Nitrobenzaldehyde and p-hydroxybenzaldehyde have no effect.

Alcohols. Nakamura's finding that ethyl alcohol has no effect on the O₂ uptake of the bacteria has been confirmed. However,

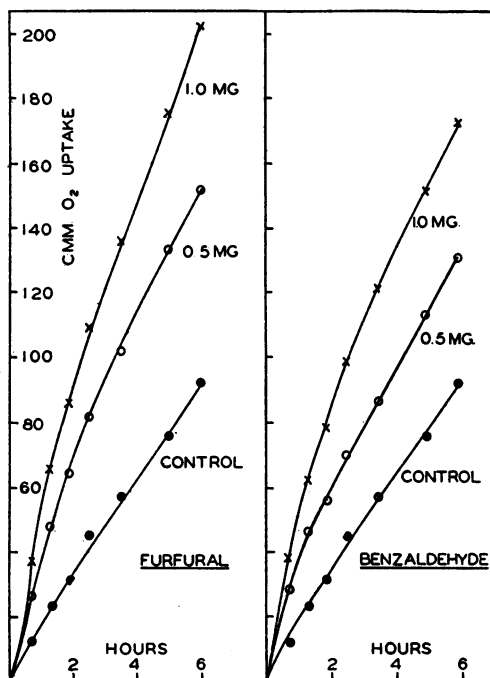


FIG 1

FIG. 1. EFFECT OF FURFURAL AND BENZALDEHYDE ON THE O₂ UPTAKE OF THE TUBERCLE BACILLUS AT pH 6.7 AND 37°

TABLE 1

Effect of 1.0 mgm. of various compounds on the O₂ uptake of two concentrations of tubercle bacilli

The uptake of the controls has been subtracted from that of the bacteria plus the compound. The values given were obtained between 5 and 6 hours. pH 6.7 and 37°.

COMPOUND	BENZALDEHYDE		ISOAMYL ALCOHOL		ACETATE		BENZOATE		SALICYLATE	
	0.3	0.6	0.3	0.6	0.3	0.6	0.3	0.6	0.3	0.6
Milliliters of bacteria...	0.3	0.6	0.3	0.6	0.3	0.6	0.3	0.6	0.3	0.6
O ₂ uptake in cmm.....	126	178	99	112	55	77	101	146	130	167

higher alcohols of the same series such as propyl, isopropyl, butyl, isobutyl, amyl and isoamyl increase the O₂ uptake. This is

shown in table 2. Capryl alcohol has no effect. Good concentration curves can only be obtained with very small amounts of the alcohols and this is shown in figure 2 for isoamyl alcohol. Increase in bacterial concentration increases the amount taken up but again this is not proportional to the number of bacteria. This is shown in table 1 for isoamyl alcohol.

TABLE 2
Effect of 0.2 mgm. of various alcohols on the O₂ uptake of tubercle bacilli at pH 6.7 and 37°

TIME		CONTROL	AMYL	ISOAMYL	BUTYL	ISOBUTYL	PROPYL	ISOPROPYL
hr.	min.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
	45	11	20	25	25	26	28	28
1	30	27	47	53	51	52	56	52
2	15	45	76	85	73	82	86	79
3	25	70	118	129	125	117	121	111
4	25	93	153	169	166	150	155	143
5	25	113	185	192	205	181	185	170
6	20	139	216	220	221	214	220	202

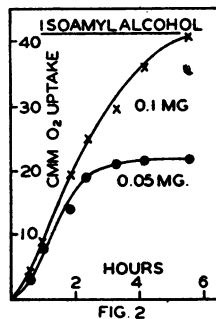


FIG. 2. EFFECT OF ISOAMYL ALCOHOL ON THE O₂ UPTAKE OF THE TUBERCLE BACILLUS AT pH 6.7 AND 37°

The uptake of the control which at the end of the experiment was 185 mm. O₂ has been subtracted from the uptake of the bacteria plus the alcohol.

Fatty acids. In confirmation of Loebel *et al.* the sodium salts of the higher fatty acids increase the O₂ uptake of the bacteria. This effect, however, is small in comparison with the effects of the lower fatty acids. The sodium salts of acetic, propionic, butyric, and valeric acids all cause definite increases in the O₂ uptake. Formate has only a slight effect. These increases are

proportional to the concentration of acid and good curves can be obtained with amounts ten times greater than the corresponding alcohols. Figure 3 shows such curves for sodium acetate. Increase in bacterial concentration causes an increase in O_2 uptake but this is not proportional (table 1).

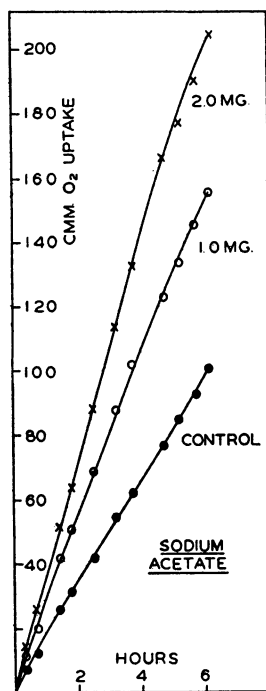


FIG. 3.

FIG. 3. EFFECT OF SODIUM ACETATE ON THE O_2 UPTAKE OF THE TUBERCLE BACILLUS AT pH 6.7 AND 37°

Salicylates and related compounds. Depending on the concentration, sodium salicylate (o-hydroxybenzoate) will more than double the O_2 uptake of the bacteria. The para isomer increases the uptake much less and the meta isomer is without effect. Acetylsalicylic acid has no effect when first added but after a latent period increases the uptake to the same extent as sodium salicylate. This can only be explained by the hydrolysis of the acetyl group. Methyl salicylate and 3,5 dinitrosalicylic acids

gave negative results (fig. 4). The increased O_2 uptake is proportional in a certain concentration range to the amount of salicylate (fig. 5). Increase in bacterial concentration increases the uptake but not proportionally (table 1). The extra O_2 uptake caused by sodium salicylate is not inhibited by either methyl salicylate or 3,5 dinitro salicylic acid.

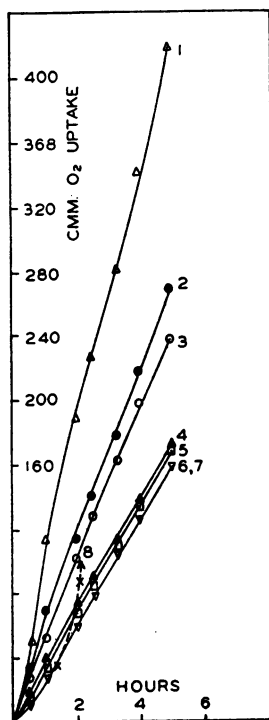


FIG. 4

Fig. 4. The effect of 1.0 mgm. on each of the following compounds on the O_2 uptake of the tubercle bacillus at pH 6.7 and 37° : (1) salicylic acid; (2) benzoic acid; (3) p-hydroxybenzoic acid; (4) control; (5) m-hydroxybenzoic acid; (6) p-aminobenzoic acid; (7) methyl salicylate; (8) acetylsalicylic acid. The acids were neutralized and made up in buffer.

Sodium benzoate also markedly increases the O_2 uptake of the bacteria (fig. 4), and this increase is a function of the concentration of benzoate. The effect of two concentrations of bacteria is shown in table 1. p-Aminobenzoic acid which seems to be a metabolite of certain bacteria (3) is without effect.

Phthallic, nicotinic, and pyrazine monocarboxylic acids gave negative results. Phenol in equivalent concentrations inhibited. The salicylates and benzoates had no effect on any of the other bacteria tested.

0.05 M KCN completely but reversibly inhibits the effects of all the compounds used.

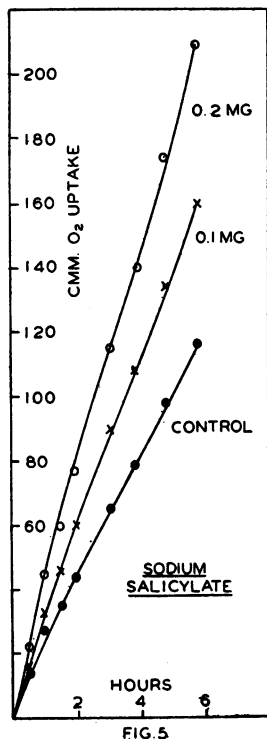


FIG. 5. EFFECT OF THE CONCENTRATION OF SALICYLIC ACID ON THE O₂ UPTAKE OF THE TUBERCLE BACILLUS AT pH 6.7 AND 37°

DISCUSSION

All the substances that have been tried give definite and clear results, i.e. in optimal concentrations an increase of 50 to 200 per cent over the control O₂ uptake of the bacteria can be obtained. The evidence indicates that the substances are acting as substrates undergoing oxidation and not as accelerators of the normal bacterial metabolism. But because of the lack of definite

end-points and because proportional concentration curves can in some cases only be obtained at rather low concentrations the possibility remains that some of these compounds may be acting as catalysts. This seems unlikely because there is no common chemical or physical property of these compounds which might influence the bacterial enzymes or the permeability of the capsule in the same way. The assumption that they are all substrates does not necessarily mean that they are normal metabolites of the cell. The results suggest the probability that substances of similar chemical constitution may be important in the normal cellular metabolism. The effect of these compounds on the growth of the bacteria is being tested. It should be added that the autoxidizability of all the compounds used was tested and found negligible under the conditions of the experiments. Further, blastomycetes showed no extra O_2 uptake in the presence of benzaldehyde and furfural and the latter had very little effect on the uptake of *Staphylococcus aureus* and *E. coli*. The H37 strain behaves in a similar way with all these compounds except furfural. This compound has a very small effect on the O_2 uptake compared to its effect on the B₁ strain.

SUMMARY

1. The inability of carbohydrate, amino acids, and other acids such as lactic, citric, and succinic to affect the O_2 uptake of the tubercle bacillus has been confirmed.
2. All the aldehydes tested increase the O_2 uptake. Furfural and benzaldehyde have the greatest effect.
3. The sodium salts of the lower fatty acids and the lower alcohols except ethyl and capryl also increase the uptake.
4. The sodium salts of salicylic and benzoic acids markedly increase the uptake. p-Hydroxybenzoic has a small effect and m-hydroxybenzoic, p-amino benzoic, 3,5 dinitrosalicylic acids and methyl salicylate have no effect. Acetylsalicylic acid is effective only after hydrolysis of the acetyl group.
5. The O_2 uptake is proportional in certain ranges to the concentration of the compound added but no definite endpoints

were obtained. The O₂ uptake is always accompanied by CO₂ production.

6. The O₂ uptake is dependent on the bacterial concentration but is not proportional to it.

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