THE EFFECT OF SODIUM FLUORIDE ON THE METABOLISM OF CERTAIN MYCOBACTERIA

ROBERT J. FITZGERALD AND FREDERICK BERNHEIM

Department of Physiology and Pharmacology, Duke University School of Medicine, Durham, North Carolina

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Little is known about the effect of fluorides on bacteria. In large concentrations inorganic and organic fluoride compounds inhibit growth. Fluorine is not known to be an essential trace element for bacteria, fungi, or algae. Recently Davis and Dubos (1947) have shown that fluoride inhibits the action of a lipase on "tween 80" in concentrations that do not affect the growth of *Mycobacterium tuberculosis* H37RV. The following is an account of the effects of the fluoride ion on the metabolism of certain mycobacteria.

EXPERIMENTAL RESULTS

Most of the experiments were done with a rapidly growing BCG strain of *M. tuberculosis* (ATC 8240). Similar effects were, however, obtained with *M. tuberculosis* (ATC 607) and the strain H37RV.[1] The BCG organisms were grown in Long's medium for 2 to 4 days. The masses were broken up and washed in water by centrifugation in a Hopkins tube. Even suspensions were made in M/20 Na-K-phosphate buffer at various hydrogen ion concentrations. Three to four mg dry weight of bacteria were used in each Warburg vessel, which contained a total volume of 2.0 ml. The oxygen uptake was measured in air at 37 C. Various samples of analytical grade sodium fluoride were used.

Figure 1 shows the effect of several concentrations of sodium fluoride on the oxygen uptake of washed suspensions of BCG at different hydrogen ion concentrations. A large increase in the rate of oxygen uptake occurs. For low concentrations of fluoride the increase is maximal at pH 6.0. At pH 6.7 a higher concentration of fluoride is necessary to produce a corresponding increase, and at pH 7.8 even high concentrations have only small effects. At pH 6.0 and 6.7, if the concentration of fluoride is too large, acceleration gives way to inhibition, but at pH 7.8 no inhibition occurs with the concentrations used. The fluoride ion is, therefore, more effective on the acid side of neutrality. The absolute increase in oxygen uptake for any concentration of fluoride depends on the number of bacteria present. Thus at pH 6.0 a concentration of fluoride that causes an increased O_2 uptake with 0.5 ml of a bacterial suspension may cause an inhibition when only 0.2 ml of suspension are used.

Equimolar solutions of sodium iodide, bromide, and chloride are without effect on the oxygen uptake. Under conditions that produce maximal acceleration with fluoride, equimolar concentrations of oxalate are without effect. This in-

[1] Obtained originally from the culture collection of the National Tuberculosis Association, Trudeau, New York.

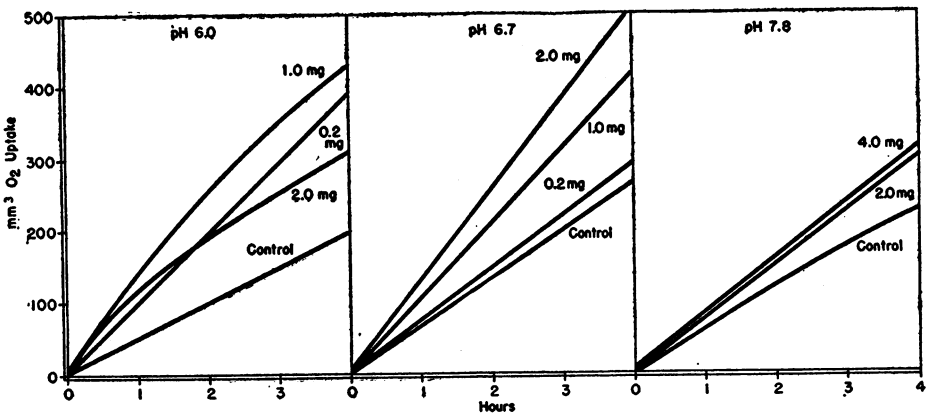

FIG. 1. THE EFFECT OF DIFFERENT AMOUNTS OF NaF ON THE OXYGEN UPTAKE OF SUSPENSIONS OF MYCOBACTERIUM BCG AT 3 HYDROGEN ION CONCENTRATIONS

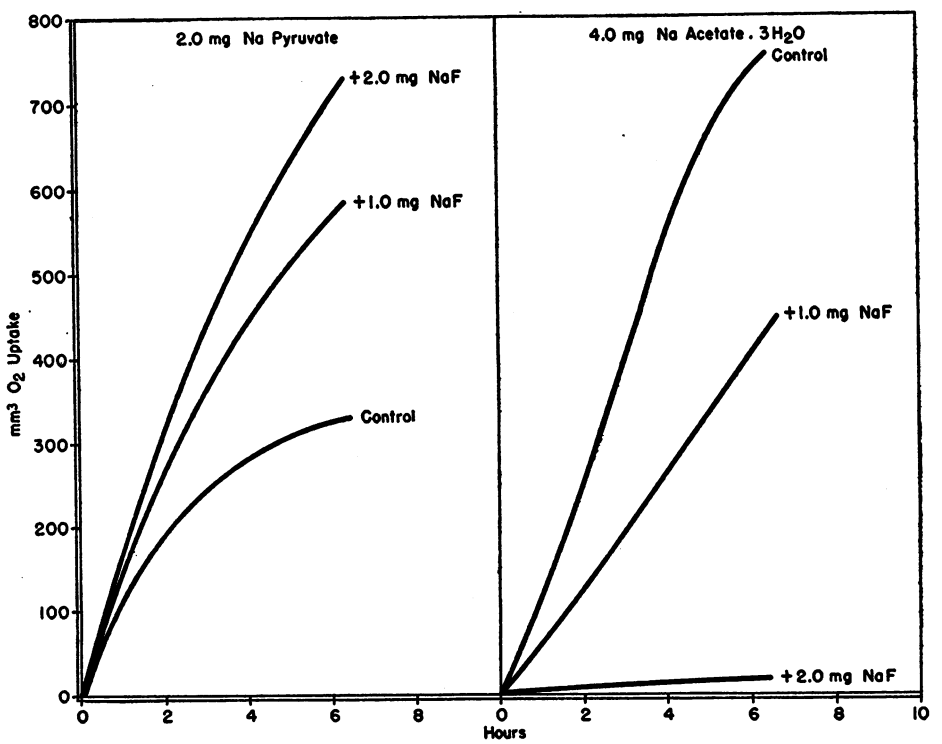

FIG. 2. THE EFFECT OF DIFFERENT AMOUNTS OF NaF AT pH 6.0 ON THE OXIDATION OF PYRUVATE AND ACETATE BY MYCOBACTERIUM BCG

The respective control uptakes, with and without fluoride, have been subtracted.

dicates that the fluoride effect is not due to the removal of Ca ions. Evidence that fluoride is accelerating an oxidative reaction in the cell is seen in the fact that the R.Q., which varies between 0.75 and 0.80 at pH 6.0, is increased to 0.85

to 0.90 in the present of fluoride. It is possible that carbohydrate oxidation is accelerated, or that the oxidation of fat is inhibited.

These possibilities were investigated by adding various carbohydrates and fatty acids to the bacterial suspensions in the presence and absence of fluoride. The

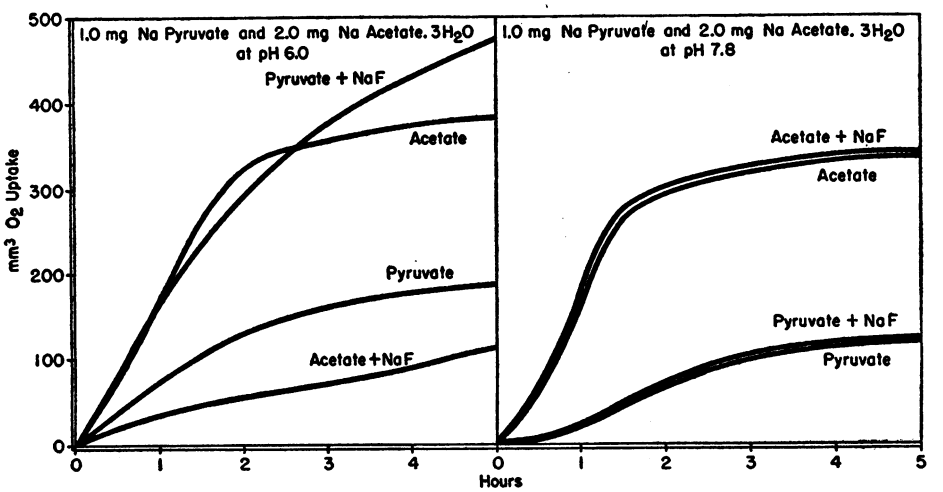

FIG. 3. THE EFFECT OF 2.0 MG NaF ON THE OXIDATION OF PYRUVATE AND ACETATE AT pH 6.0 AND 7.8 BY MYCOBACTERIUM BCG

The respective control uptakes, with and without fluoride, have been subtracted.

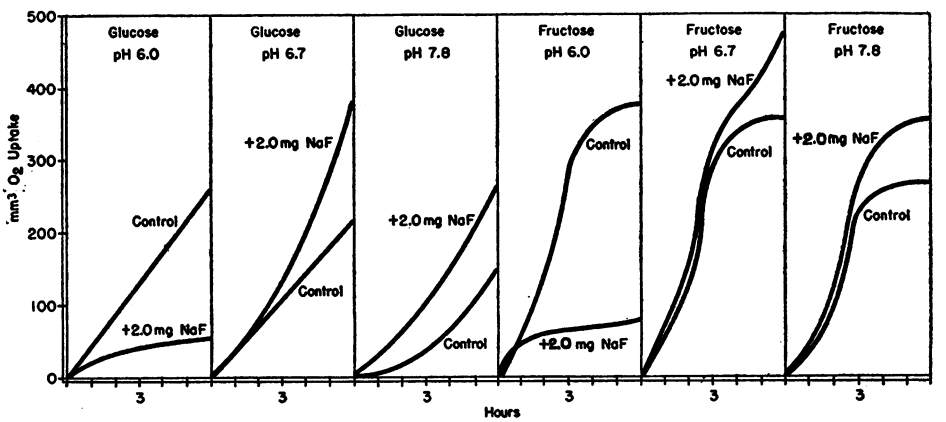

FIG. 4. THE EFFECT OF 2.0 MG NaF ON THE OXIDATION BY MYCOBACTERIUM BCG OF GLUCOSE AND FRUCTOSE AT 3 HYDROGEN ION CONCENTRATIONS

The respective control uptakes, with and without fluoride, have been subtracted.

results are shown in figures 2 to 5. At pH 6.0 in the presence of fluoride both the rate of oxidation of pyruvate and the absolute O_2 uptake are increased. The maximum effect occurs with 1.0 to 2.0 mg of fluoride, whereas the maximum effect on the control uptake is obtained with 1.0 mg or less. This may mean that a system more sensitive than pyruvate to fluoride is present in the cell. Under the same conditions, fluoride, depending on its concentration, has no effect or in-

hibits the oxidation of acetate. Pyruvate, therefore, cannot be oxidized to acetate by these bacteria but probably condenses with a metabolite in the cell and then is oxidized. Such a condensation probably also occurs during the oxidation of pyruvate in the absence of fluoride, since the oxygen uptake stops when 1 to 2 atoms of oxygen are taken up. If acetate were an intermediate product, the oxidation would proceed to completion because acetate is oxidized under the same conditions to CO_2 and H_2O. Decarboxylation, however, accompanies the oxidation of pyruvate because the R.Q. rises to 1.1 to 1.2 in the absence of, and to 0.96 to 1.1 in the presence of, fluoride.

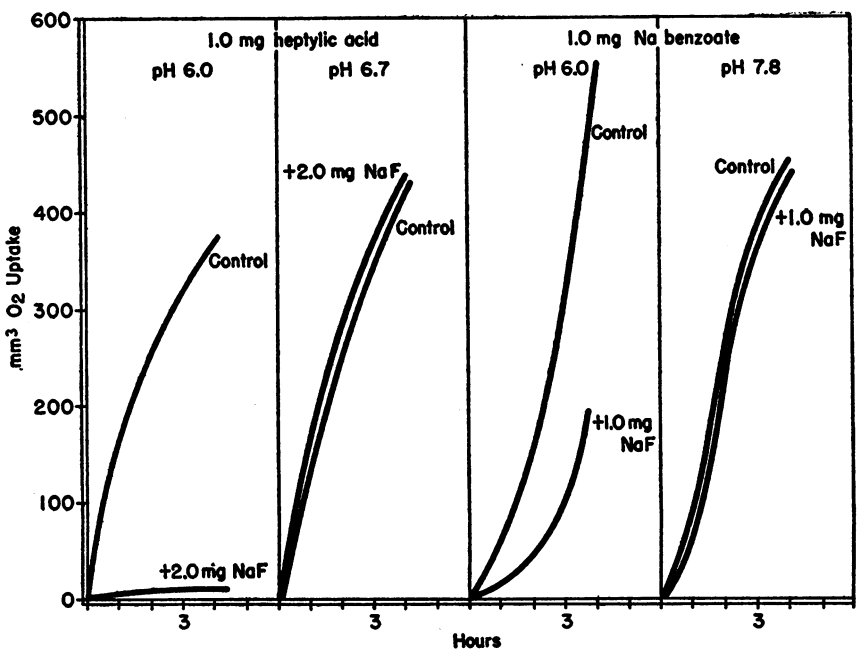

FIG. 5. THE EFFECT OF NaF ON THE OXIDATION BY MYCOBACTERIUM BCG OF HEPTYLIC AND BENZOIC ACIDS AT DIFFERENT HYDROGEN ION CONCENTRATIONS

The respective control uptakes, with and without fluoride, have been subtracted.

At pH 6.0 the oxidation of *n*-heptylic, oleic, and benzoic acids is inhibited by fluoride. The oxidation of lactate is accelerated in the same way as that of pyruvate, probably because it is readily oxidized to pyruvate. At pH 6.0 the oxidation of glucose, fructose, and trehalose is inhibited by fluoride, but at pH 6.7 or 7.8 a moderate acceleration occurs.

The effect of various concentrations of fluoride on the rate of growth of *Mycobacterium* BCG was investigated using tween medium (Dubos *et al.*, 1946) buffered at pH 7.0 and pH 6.0 The effect of adding 50 mg per cent lactate to these media was also investigated. The inoculum for each tube containing 10 ml of medium consisted of 0.5 ml of a 1 to 2 dilution of a 48-hour culture grown in tween medium at pH 7.0 and washed and resuspended in medium of the appropriate

pH. Growth was followed nephelometrically by means of the Evelyn photoelectric colorimenter using a 660 filter. At pH 7.0 the growth rate is unaffected by fluoride, or lactate, or a combination of both. At pH 6.0 a different picture appears. The control rate of growth is considerably less than at pH 7.0. In addition, the growth rate is further diminished by 5.0 and 10.0 mg per cent NaF, and this inhibitory effect on growth is potentiated in the presence of 50 mg per cent lactate. This concentration of lactate is not inhibitory in the absence of

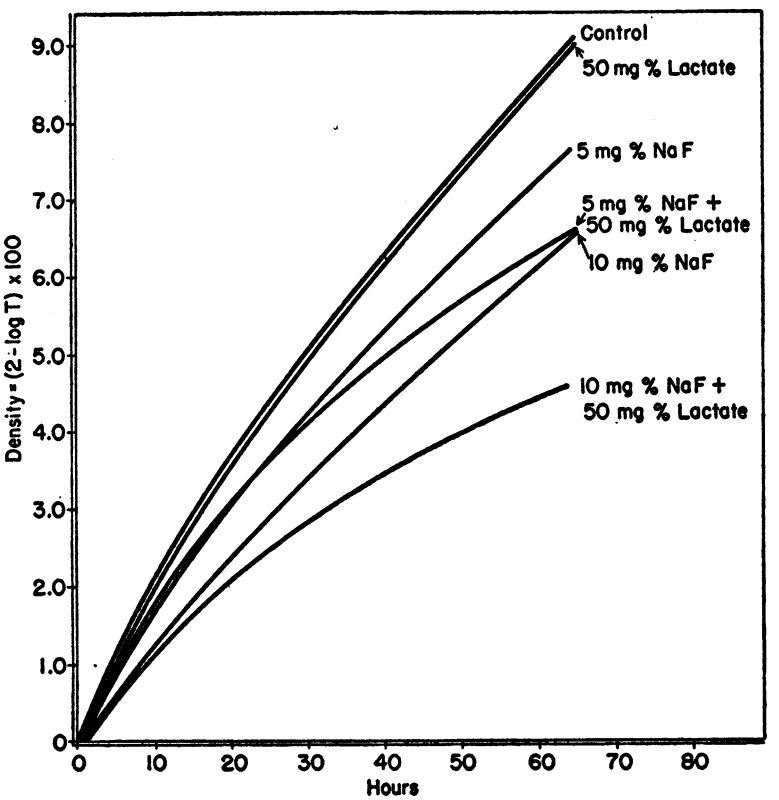

FIG. 6. THE EFFECT OF NaF WITH AND WITHOUT LACTATE ON THE GROWTH OF MYCOBACTERIUM BCG IN DUBOS MEDIUM AT pH 6.0

fluoride. These facts are shown in figure 6. The pH remained constant throughout the experiments. No inhibition of growth was observed with 1.0 mg per cent NaF either in the presence or absence of lactate.

SUMMARY

The oxygen uptake of washed suspensions of *Mycobacterium* BCG is greatly increased by sodium fluoride. The amount of the increase depends on the relative concentrations of fluoride and bacteria and upon the hydrogen ion concentration.

The effect is greatest at pH 6.0, intermediate at pH 6.7, and least at pH 7.8. At pH 6.0 the increased uptake is accompanied by a rise in R.Q.

At pH 6.0 the oxidation of pyruvic acid and lactic acid is increased by fluoride, both in rate and amount. The effect diminishes at lower hydrogen ion concentrations.

At pH 6.7 and 7.8 the oxidation of glucose, fructose, and trehalose is increased by fluoride. At pH 6.0 fluoride inhibits the oxidation of these sugars.

At pH 6.0 the oxidation of acetic, heptylic, oleic, and benzoic acids is inhibited by fluoride. At lower hydrogen ion concentrations fluoride has no effect.

At pH 6.0, 5 to 10 mg per cent fluoride inhibits growth, and the presence of lactate increases the inhibition. At pH 7.0 there was no inhibition by fluoride either in the presence or absence of lactate.

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