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Stimulation of respiratory activity of Shigella sonnei by certain nitrogenous compounds*

by

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Shigella sonnei has been reported to require nicotinic acid (NAKAMURA and PITSCH, 17). The nicotinic acid requirement of shigellae have been reported by numerous workers (KOSER *et al.*, 7, 8, 9; KLIGLER and GROSOWITZ, 5; SAUNDERS *et al.*, 18; WEIL and BLACK, 20; MIZUNO and KOSAKA, 12, 13). However, strains of shigellae which could grow in the absence of nicotinic acid have been reported (KOSER and WRIGHT, 10; MIZUNO and KOSAKA, 12). The effects of nicotinamide on the respiratory activity of dysentery bacteria have been studied by DORFMAN *et al.* (2) and by SAUNDERS *et al.* (18).

In the present investigation we have studied the effects of nicotinic acid and nicotinamide on the respiratory rate of *S. sonnei.* In addition, pyridine-3-sulfonic acid was studied since it has been reported as a growth factor for shigellae (MIZUNO and NOJIMA, 14; MIZUNO and TAMURA, 15; NAKAMURA and PITSCH, 16) and other organisms (LWOFF and QUERIDO, 11). We included tryptophan in our studies because it is one of the possible precursors of nicotinic acid. Glutamic acid, which MIZUNO and KOSAKA (12) found to be important in the growth of *S. dysenteriae,* was also tested. Aspartic acid was found to yield maximum growth of *S. flexneri* in synthetic media of a number of nitrogenous sources tested (ERLANDSON and MACKEY, 3), so it was assayed. In addition, asparagine, thiamin, glutamine, glycine, and arginine were studied for their effects on the respiratory activity of *S. sonnei.*

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MATERIALS AND METHODS

Strain 5044-59 which was obtained from Dr. W. H. Ewing, Enteric Bacteriology Unit, Microbiology Section, Communicable Disease Center, Public Health Service, Chamblee, Georgia, was used throughout these experiments. The organism was repeatedly checked morphologically, biochemically, and serologically (Lederle Laboratories Division, Group D Shigella grouping sera) for purity. Cultures grown at 37°C on nutrient agar (Difco) slants for 48 hours formed the stock strains and were stored at 4°C. These cultures were transferred to fresh media every 4 weeks. Cultures for experimental work were maintained in nutrient broth at 37°C and transferred daily. Cells for manometric studies were prepared in the following manner: (a) 24-hour cultures grown in nutrient broth (500 ml flasks) were harvested; (b) the cells were washed three times by centrifugation in buffered saline; (c) the cells were resuspended in buffered saline to obtain the desired cell concentration (approximately 10^{10} cells/ml). Plate counts were performed to determine the population of the cell suspensions used in the manometric experiments. Conventional manometric methods were employed (UMBREIT *et al.*, 19). Each flask received 1.0 ml of washed cells, 1.0 ml of glucose substrate (0.15M), and 1.0 ml of substance being tested for its stimulatory activity upon glucose oxidation. Most of the reagents used in the experimental phase were purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A.

The data were recorded as μl of oxygen taken up every 15 minutes for a period of 120 minutes at 37°C. The data which were plotted on the graphs have the respiratory rate due to the glucose substrate subtracted from the total activity. This showed a better comparison of the effects of the various test substances on the oxygen uptake of glucose by *S. sonnei.*

RESULTS

Figure 1 shows the stimulatory activity of nicotinic acid and nicotinamide upon the respiratory activity of *S. sonnei.* Note the delay in the stimulation of respiration for a period of approximately 30 minutes. Maximum stimulation was obtained with 1000 μg/ml of nicotinic acid; 10 μg/ml of nicotinic acid (the concentration at which it is present in the chemically defined medium of ERLANDSON and MACKEY, 3) produced less stimulation. On the other hand nicotinamide was far more active in its ability to stimulate oxygen uptake by the *Shigella.* Maximum stimulation was produced by 10 μg/ml, a rate almost as high as 1000 μg/ml of nicotinic acid. Even 0.1 μg/ml nicotinamide produced more respiratory rate stimulation than 10 μg/ml of nicotinic acid.

Tryptophan, which could partially replace the nicotinic acid requirement of *S. sonnei* in chemically defined media (NAKAMURA and PITSCH, 16), caused about a 40% stimulation of oxygen uptake compared to nicotinic acid (figure 2).

Pyridine-3-sulfonic acid stimulated the respiratory rate of the cells; in fact, it almost doubled the rate compared to maximum stimulation produced by nicotinic acid (figure 3). Aspartic acid produced maximum stimulation of all of the compounds tested (figure 4). Thiamin, glutamic acid, and asparagine were also highly stimulatory. The other agents tested produced little or no increase in the respiratory rate of *S. sonnei.*

DISCUSSION

Our results agree in general with the conclusions of DORFMAN *et al.,* (2) and SAUNDERS *et al.* (18). They found that nicotinic acid, nicotinamide, and cozymase stimulated the respiration of glucose-utilizing *Shigella sonnei.* They found that nicotinamide stimulated the oxygen consumption more than nicotinic acid; our findings were the same, in terms of concentrations involved. For example, 10 μg/ml of nicotinamide produced almost as much stimulation of respiration as 1000 μg/ml of nicotinic acid. However, as far as total stimulation was concerned, nicotinic acid exerted the greater stimulatory activity (by a slight margin).

Tryptophan may have owed its stimulatory activity directly or indirectly to being first converted to nicotinic acid. Tryptophan is a postulated precursor of nicotinic acid (HUNDLEY, 4). The lower activity of tryptophan compared to nicotinic acid could conceivably be due to the limiting factor of rate nicotinic acid biosynthesis.

The stimulatory activity of pyridine-3-sulfonic acid upon the oxidation of glucose by *S. sonnei* was not surprising since MIZUNO and NOJIMA (14) found that this substance was a growth accessory factor for *S. dysenteriae.* Furthermore, MIZUNO and TAMARA (15) found that pyridine-3-sulfonic acid could be substituted for nicotinamide in the growth of *Staphylococcus aureus.*

Pyridine-3-sulfonic acid was reported to be a growth factor for other organisms by LWOFF and QUERIDO (11). On the other hand, pyridine-2-sulfonic acid was devoid of growth-promoting activity in the dysentery bacillus (DORFMAN *et al.,* 1). A possible explanation for this stimulatory activity of the pyridine-3-sulfonic acid is that the sulfonic acid group is removed by the organisms and then the pyridine is carboxylated to produce nicotinic acid. Another explanation is that pyridine-3-sulfonic acid might be converted into the coenzymes (in place of nicotinic acid) without altering the activity of the unnatural coenzymes.

Aspartic acid stimulated the respiration of *S. sonnei* more than any other compound tested. ERLANDSON and MACKEY (3) reported that aspartic acid produced best growth of *S. flexneri* of the 34 nitrogenous sources they studied. Thiamin also stimulated the respiratory rate more than many of other nitrogenous compounds tested. KLIGLER *et al.* (6) found that thiamin increased the utilization of glucose (in the presence of nicotinic acid) by *Staphylococcus aureus.* They postulated that thiamin acts as a catalyzer in the oxidation of pyruvic acid. The

role of glutamic acid in the nutrition of *S. dysenteriae* was studied by MIZUNO and KOSAKA (13) and they found that it was an indispensable nitrogen source. YEE *et al.* (21, 22) studied the oxidation of glutamate by *S. flexneri* and found that the organisms oxidized glutamate at a fairly rapid rate. In our studies glutamic acid stimulated the oxidation of glucose by *S. sonnei.*

We do not yet know the mechanisms by which the various nitrogenous substances stimulated the respiration of *S. sonnei.* It would be interesting to determine whether the stimulation of respiration can be correlated to growth rates *in vitro.* Furthermore, the role of the increased respiratory rate upon the pathogenicity of organisms would make an interesting study. Currently, we are studying the effects of ultraviolet light upon the respiratory activity of *S. sonnei* to determine if certain enzymes are more susceptible to irradiation than others.

SUMMARY

Nicotinic acid, nicotinamide, tryptophan, pyridine-3-sulfonic acid, aspartic acid, thiamin, glutamic acid, and asparagine stimulated the respiratory activity of *Shigella sonnei.*

RESUMEN

Las siguientes sustancias: ácido nicotínico, nicotinamida, triptofano, ácido piridin-3-sulfónico, ácido aspártico, tiamina, ácido glutámico y asparagina son capaces de estimular la respiración de *Shigella sonnei* en presencia de glucosa; el ácido aspártico es el más efectivo de ellas.

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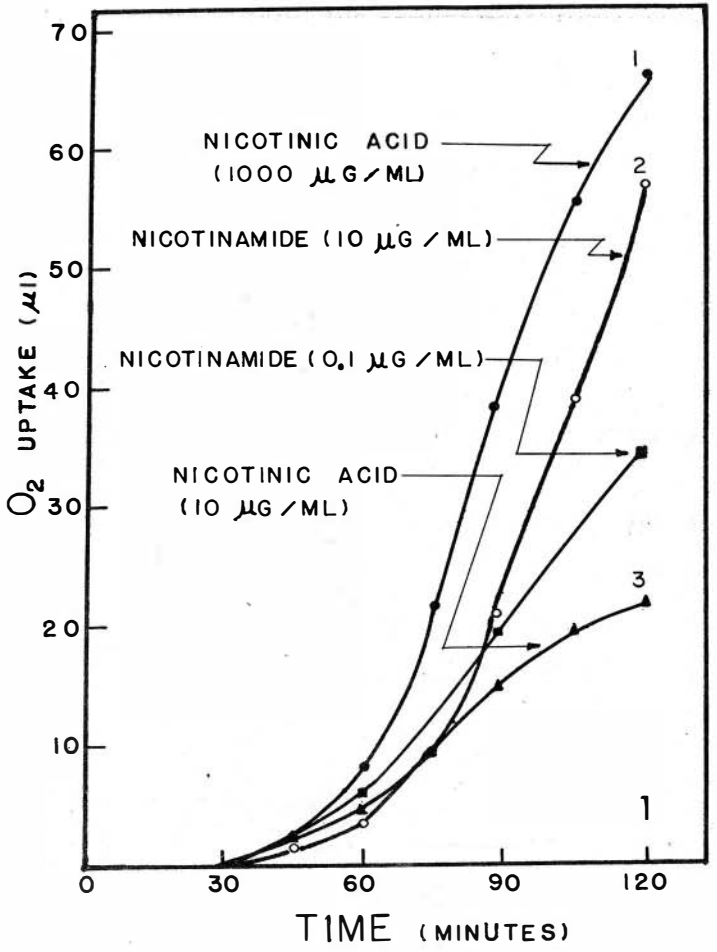
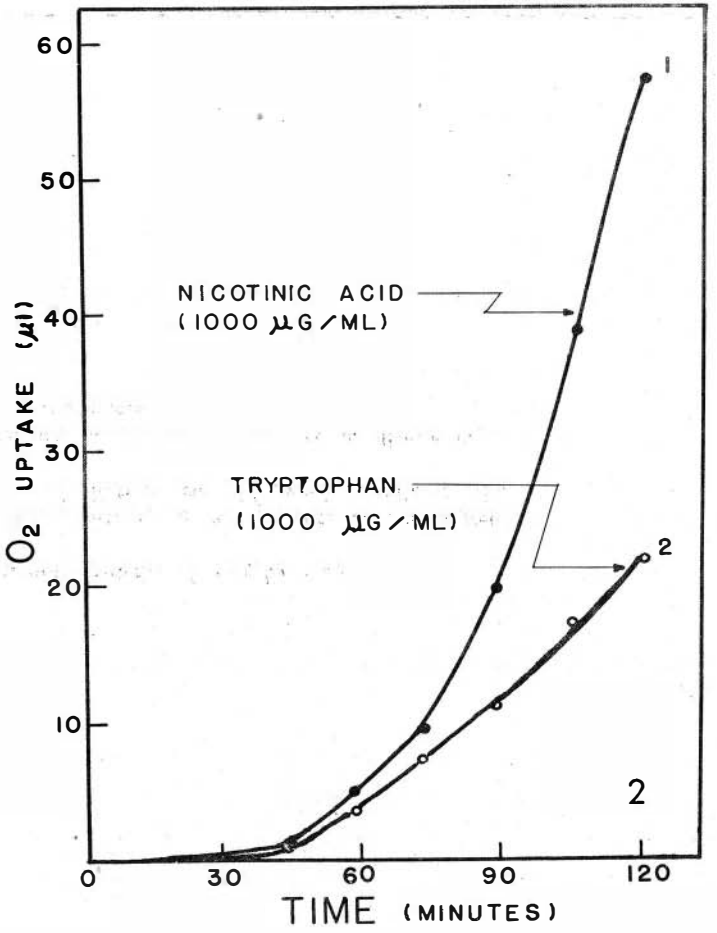
Figs. 1-2. Respiratory patterns of *Shigella sonnei.*

Fig. 1. Respiratory activity in the presence of two concentrations of nicotinic acid and nicotinamide.

Fig. 2. Respiratory activity in the presence of equal concentrations of nicotinic acid and tryptophan.

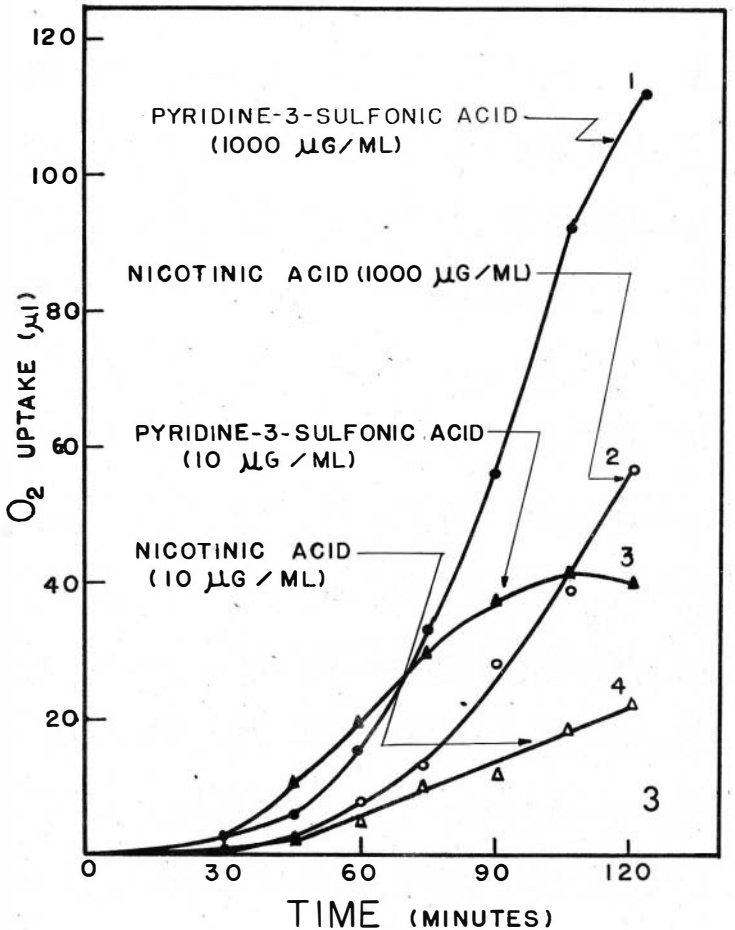

NICOTINIC ACID
(1000 µG/ML)
NICOTINAMIDE (10 µG/ML)
NICOTINAMIDE (0.1 µG/ML)
NICOTINIC ACID
(10 µG/ML)
1
2
3
O2 UPTAKE (µl)
TIME (MINUTES)

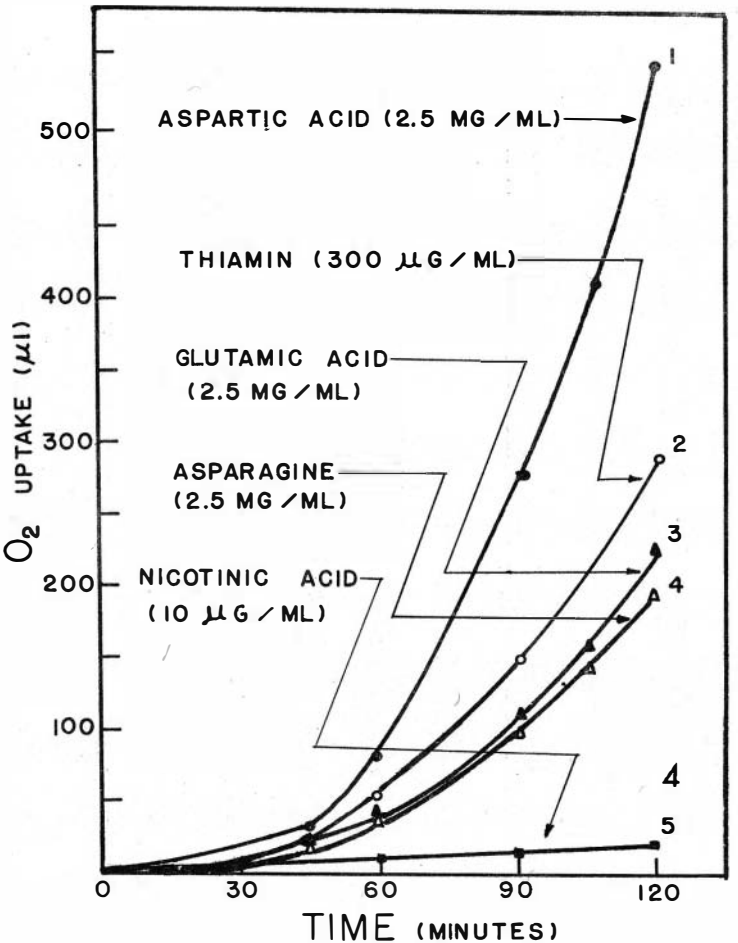

NICOTINIC ACID
(1000 µG/ML)
TRYPTOPHAN
(1000 µG/ML)
1
2
O2 UPTAKE (µl)
TIME (MINUTES)

Figs. 3-4: Respiratory patterns of *Shigella sonnei*.

Fig. 3: Respiratory activity in the presence of two concentrations of nicotinic acid and pyridine-3-sulfonic acid

Fig. 4: Respiratory activity in the presence of diverse nitrogenous compounds.


120
100
80
60
40
20
O_2 UPTAKE (µl)
PYRIDINE-3-SULFONIC ACID
(1000 µG/ML)
NICOTINIC ACID (1000 µG/ML)
PYRIDINE-3-SULFONIC ACID
(10 µG/ML)
NICOTINIC ACID
(10 µG/ML)
1
2
3
4
3
0
30
60
90
120
TIME (MINUTES)


500
400
300
200
100
O_2 UPTAKE (µl)
ASPARTIC ACID (2.5 MG/ML)
THIAMIN (300 µG/ML)
GLUTAMIC ACID
(2.5 MG/ML)
ASPARAGINE
(2.5 MG/ML)
NICOTINIC ACID
(10 µG/ML)
1
2
3
4
4
5
0
30
60
90
120
TIME (MINUTES)