

found are less than 5 microns, averaging around 1 to 3 microns according to several investigators. Although we have attempted to establish a solubility correction by experiments on fine quartz, so far we have not been successful.

Total silica was determined by the usual gravimetric hydrofluoric acid method. Essentially the free silica method consisted in digesting the ash residues at room temperature with hydrofluosilicic acid and then determining the undissolved free silica by the hydrofluoric acid method.

Table I indicates data and results on 14 silicotic lungs obtained from autopsies in Buffalo and vicinity. More cases would have been desirable but were not available. The table is largely self-explanatory.

The results show that free silica varies greatly both in actual concentration and in percent of total silica; concentrations range from 0.086% to 1.296% of the dry lung; as a part of the total silica the free silica ranges from 3.25% to 91%. The variations are not out of harmony with the fact that different persons are subjected to breathing siliceous dusts of greatly varying compositions in silica and silicates.

It is to be noted that in 5 cases (Nos. 22, 24, 25, 29, 30) the minimum concentration of free silica is within the generally accepted maximum limit of 0.20% of total silica for normal lungs (on the dry basis). The total silica, however, in these cases is well above the normal.

11126

Carbohydrate Metabolism of *Oidium lactis* and *Bacillus subtilis* in Complex Carbohydrate-rich Culture Medium.*

THEODORE E. FRIEDEMANN.

*From the Laboratory of Chemical Bacteriology, Department of Medicine,
University of Chicago, Chicago.*

In previous papers the writer has shown that the rapid growth of pneumococci¹ and streptococci² in the usual laboratory media results in a large yield of lactic acid; a small part of the sugar is further

* This study was supported by the Bartlett Memorial Fund and the Douglas Smith Foundation for Medical Research of the University of Chicago. The work was carried out with the assistance of Thaddeus C. Kmiecik.

¹ Friedemann, T. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 505.

² Friedemann, T. E., *J. Biol. Chem.*, 1939, **130**, 757.

TABLE I.
Metabolism of Bacteria in Complex Carbohydrate-rich Culture Medium.
Culture medium, meat extract (0.3%), 1% of Bacto- or Witte-peptone, 1 to 1.8% of $\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$, and 0.9% of dextrose.
Initial pH 7.6. Temperature 37.5°. Incubated 10 to 24 hours, except *C. diptheriae* which was incubated 72 hours.

Organism	Culture medium batch*	Glucose x 2 mm C ₃ per l.	Lactic acid mM per l.	Formic acid mM per l.	Acetic acid mM per l.	Ethyl alcohol mM per l.	Undeter- mined non- volatile acidity cc N per l.	Yield of lactic acid %
<i>Eberthella typhosa</i>	B ₂	44.9	15.1	26.7	11.0		-3.2	34
<i>Shigella paradysenteriae</i> (Flexner)	B ₂	42.4	14.6	20.5	12.0		0.8	34
<i>Oidium lactis</i>	B ₂	52.0	58.8	8.5	3.5		1.0	75
<i>Eberthella typhosa</i>	B ₃	51.6	14.7	29.5	17.2	15.2	3.2	29
<i>Oidium lactis</i>	B ₃	48.3	34.1	11.4	6.6	5.3	0.9	71
<i>Bacillus subtilis</i> A	B ₃	52.3	36.4	10.5	5.9	6.2	0.3	70
" B	B ₃	55.2	38.0	10.5	5.5	6.6	-1.6	69
<i>Corynebacterium diptheriae</i> (Park VIII)	B ₃	21.8	13.4	3.2	4.0	0	2.1	62
<i>Lactobacillus helveticus</i> (casei ϵ)	B ₃	71.9	64.1	0.3	0.7	1.9	3.6	89

* Date of inoculation: B₂, July 5, 1932; B₃, January, 1939. B₂ contained Bacto-peptone; B₃ contained Witte-peptone.

broken down into 2 moles of formic acid and 1 mole each of acetic acid and ethyl alcohol as in the case of *Eberthella typhosa*.^{1, 3} For the first time it was demonstrated that *Escherichia coli* may yield a maximum of 2 moles of formic acid.¹ In the present paper it will be shown that the metabolism of *Oidium lactis* (from milk and dairy products) and *B. subtilis*, the "hay bacillus", both non-pathogenic microorganisms which differ widely morphologically, follows the same pattern.

The bacteriological and analytical methods were the same as described in previous papers.⁴ The composition of the culture medium, the temperature, and the duration of incubation are shown at the top of Table I. For the purpose of comparison results are also given from a few other microorganisms which were grown in the same batch of culture medium.

The metabolism of *Eberthella typhosa* and *Shigella paradysenteriae* in culture medium B₂ resulted in a yield of 34% of lactic acid on the basis of the sugar consumed. Slightly less lactic acid was produced by *Eberthella typhosa* in medium B₃. The yield of lactic acid from *Oidium lactis* was high and approximately the same in both media, namely, 75 and 71%. *B. subtilis* yielded 70% of lactic acid in medium B₃, on the basis of the sugar consumed. Another strain of *B. subtilis* gave almost identical yields of products. Both microorganisms converted about one-fifth of the sugar into volatile products which appeared in the medium in approximately the ratio of 2 moles of formic acid to 1 mole each of acetic and ethyl alcohol. Thus in medium B₃, *Oidium lactis* produced 11.4 mM of formic acid, 6.6 mM of acetic acid, and 5.3 mM of ethyl alcohol. *B. subtilis* produced 10.5 mM of formic acid, 5.9 mM of acetic acid, and 6.2 mM of ethyl alcohol. This should be compared with the results from *Eb. typhosa*. From approximately the same quantity of sugar, *Eb. typhosa* yielded 29.5 mM of formic acid, 17.2 mM of acetic acid and 15.2 mM of ethyl alcohol; approximately 60% of the sugar was thus converted into volatile products. Succinic acid was not produced in measurable quantities in either of the media, as measured by the "undetermined non-volatile acidity."

It is evident that these products were produced by anaerobic mechanisms. Under the experimental conditions, even with a long exposure and with slow growth, a high yield of lactic acid probably would have been obtained from these organisms. Such a condition obtained in a culture of *C. diphtheriae*. Although the inoculum was

³ Harden, A., *J. Chem. Soc.*, 1901, **79**, 610.

⁴ Friedemann, T. E., *J. Bact.*, 1938, **35**, 527.

large, growth was very slow and appeared to occur chiefly at the surface. The contents of the bottle were rotated frequently during a period of 72 hours. Despite the obviously aerobic conditions, a yield of 62% of lactic acid was obtained. The yield of volatile products was similar to that obtained in aerated cultures of *Diplococcus pneumoniae*.¹ Neither *Oidium lactis* nor *B. subtilis* produced the maximum of lactic acid from sugar by growth in this medium. The yield of 89% of lactic acid in a culture of *Lactobacillus helveticus* (*casei* ϵ , v. Freudenreich) perhaps represents the maximum which could be obtained by the growth of microorganisms in this batch of culture medium.

Summary. *Oidium lactis* and *B. subtilis* were inoculated into culture media prepared from 0.3% solution of meat extract or an infusion of beef muscle, to which were added 1% of Witte peptone, 1 to 1.8% of $\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$ and 0.9% of dextrose. Both microorganisms grew rapidly at 37.5° and both yielded about 70% of lactic acid on the basis of the sugar consumed. Approximately one-fifth of the C_3 -intermediates from the sugar was further metabolized into 2 moles of formic acid and 1 mole each of acetic acid and ethyl alcohol. Their metabolism in the same culture medium is compared with that of *Eberthella typhosa*, *Shigella paradysenteriae*, *Lactobacillus helveticus* (*casei* ϵ) and *Corynebacterium diphtheriae*.

11127 P

Pantothenic Acid and Nicotinic Acid as Essential Growth Substances for Morgan's Bacillus (*Proteus morganii*).

MICHAEL J. PELCZAR, JR., AND J. R. PORTER. (Introduced by Wm. M. Hale.)

From the Department of Bacteriology, College of Medicine, State University of Iowa, Iowa City, Iowa.

Following the announcement by Fildes¹ that nicotinic acid was found to be an essential accessory nutrient for 10 strains of *Proteus*, an investigation was undertaken in this laboratory to determine the nutritional requirements of some 240 strains of *Proteus vulgaris* and other species of the same genus. During the course of the study² it was observed that all of the *Proteus morganii* strains exhibited a

¹ Fildes, P., *Brit. J. Exp. Path.*, 1938, **19**, 239.

² Pelczar, M. J., and Porter, J. R., *J. Bact.*, in press.