THE PRODUCTION OF VOLATILE FATTY ACIDS BY BACTERIA OF THE DYSENTERY GROUP.

By HARPER F. ZOLLER and W. MANSFIELD CLARK.

(From the Research Laboratories of the Dairy Division, Bureau of Animal Industry, United States Department of Agriculture, Washington.)

(Received for publication, October 11, 1920.)

INTRODUCTION.

In the course of an investigation of bacteria of the dysentery group we had occasion to determine the volatile fatty acids produced by these bacteria under different conditions. We believe the results to have considerable significance but their interpretation had best wait upon the development of more fundamental knowledge of anaerobiosis than we now possess. We therefore desire to place the following data on record without comment.

Organisms Studied—The cultures were secured from several investigators and institutions and represented the usual types together with some atypical organisms. A laboratory fire destroyed the history of each culture, but they have been separated into Shiga and non-Shiga types by mannite fermentation tests through the cooperation of Dr. J. M. Sherman of this laboratory. Fermentation data were at hand which would permit the differentiation of the non-Shiga cultures into Flexner, Strong, and other strains; but this was not done, since the results of the volatile acid work are in such close agreement that, as conducted, they have no important bearing upon the classification of the dysentery bacteria. The results from one each of typhoid (Ty Round) and paratyphoid (P Ty 16) cultures are included because of the relation existing between the dysentery and these groups of organisms. Following the laboratory number will be found the strains studied: D_1, Flexner; D_2, Shiga; D_3, Shiga; D_4, Flexner; D_5, Shiga; D_6, non-Shiga; D_8, non-Shiga; D_{10}, non-Shiga; D_{11}, non-Shiga; D_{13}, non-Shiga; D_{17}, non-Shiga; D_{23}, non-Shiga; D_{24}, Shiga; D_{33}, non-Shiga; D_{37}, Shiga.

Methods.

Media.—The medium for the growth of the organisms consisted of 1.0 per cent of "Difco" peptone, and 0.5 per cent of $Na_2HPO_4 \cdot 2H_2O$, with or without 1.0 per cent of Merck's "highest purity" dextrose. The reaction of this medium after sterilization always lay between pH 7.1 and 7.2. The hydrogen ion concentrations of the media and cultures were determined colorimetrically.

Cultures.—The organisms were inoculated into sterile 10 cc. portions of the media and incubated at 37°C. for 18 hours. These virile cultures were then poured into 990 cc. of the sterile media contained in 2 liter Florence flasks and incubated at 37°C. for the period of time indicated. The pouring was conducted under conditions which reduced the chance for contamination by this method. No control plates were run to determine this factor, since it was held that the bulky inoculation was sufficient to take care of any slight contamination should such occur. The regularity in the content of volatile fatty acids leads us to believe that there was no contamination. It was found that the maximum production of total volatile acids in sugar medium under these conditions occurred shortly before the first 12 hour period and the total quantity remained quite stationary during the following 60 hours (Fig. 1). Accordingly, the cultures were allowed to become 48 hours old merely as an arbitrary period. The cultures in non-sugar media were allowed a longer period before examining for volatile acids, because volatile bases were investigated in the same cultures. It is barely possible that the values for the volatile acids obtained in the case of these cultures grown for a longer period of time are somewhat too low because of the chance of volatile acid utilization.

Anaerobic Cultures.—"Anaerobic" conditions were obtained by evacuation of the inoculated flasks with a mercury-vapor pump (a single Kraus modeled pump) in series with a motor-driven Geryke vacuum pump. Gum stoppers, bearing a thick-walled tube drawn out to a narrow neck to permit sealing with a fine tipped flame, were cemented while hot with a heavy rubber-rosin cement into the necks of the flasks. These stoppers had been previously sterilized, and the glass tubes contained a plug of cotton to prevent contamination of the

flasks while evacuating. After pumping out the bulk of the air with the Geryke pump till the Plücker tube discharge showed a declining spark, the mercury-vapor pump was started and it was allowed to pump for 20 minutes more or until the Plücker tube discharge showed a continued decline in brilliancy. The flasks were sealed off while the pumps were in operation.

Separation of the Volatile Acids from the Cultures.—10 per cent sulfuric acid was added to the cultures until a drop of thymol blue showed a distinct red; this represented a pH of 1.0 or less. Steam formed by boiling distilled water in the presence of barium hydroxide was led into the flasks holding the cultures. The flasks in turn were connected to vertical condensers through which ice-cooled water circulated. The condensers were arranged vertically to facilitate the delivery of the condensed fatty acids. A small piece of paraffin was placed in the cultures to overcome frothing during distillation. The volume of liquid in the culture flasks was held constant by means of a screened flame. Fractions of 500 cc. each were caught and titrated with 0.1 N NaOH to phenolphthalein. The distillation was continued until the last fraction required no more than 2 cc. of 0.1 N NaOH. Usually ten to fourteen fractions were collected. These were evaporated *in toto* and made up to a definite volume.

Distillation was conducted upon a like volume of sterile medium to determine the factor for carbon dioxide, etc.

Quantitative Analysis of the Volatile Fatty Acids.—The modified Duclaux method published by Gillespie and Walters[1] was followed in the main in the determinations. It was found advisable to determine new constants for formic, acetic, propionic, and normal butyric acids with the distillation apparatus employed. The still was of Jena glass throughout and contained a small, closed end, inverted tube in the solution to insure against excessive spattering and to produce a steady flow of vapor. The constants found did not depart radically from those obtained by Gillespie and Walters. The still flask was heated on the sides and bottom with electrically heated coils.

An aliquot of the total volatile acid concentrate was employed which contained from 60 to 80 cc. of 0.1 N volatile acids.

[1] Gillespie, L. J., and Walters, E. H., *J. Am. Chem. Soc.*, 1917, xxxix, 2027.

The mode of treating the results depended somewhat upon the number of volatile acids found to be present in the cultures under examination. For only two acids the algebraic method[1] (page 2036) was used, although the graphical method (page 2040) was found to be somewhat more rapid. When three acids were found to be present the graphical method mentioned by Gillespie and Walters (page 2054) was followed. This latter method involved much less calculation than the algebraic and was equally accurate.

As a check on the modified Duclaux method, the formic acid was determined in several cultures by the mercuric chloride method of Franzen and Egger.[2] The two methods gave values for this acid harmonizing within 2 per cent of the whole. It is assumed that the values found for the other acids are as accurate.

In the cultures containing butyric acid its presence was further established by preparing the quinine salt as outlined by Phelps and Palmer[3] and determining its melting point. The distillation values accurately establish the identities of the acids, provided the manipulations are carefully conducted and the constants are determined with purified acids.

Sugar determinations were conducted upon the media and several cultures to obtain data regarding the utilization of the sugar and the amount of acid formed therefrom. From 30 to 40 per cent of the sugar was utilized by the bacteria in cultures 2 and 14 days old. It was at first feared that, because the total quantity of volatile acids produced by each organism in the same length of time reached about the same value, not enough glucose was present. It is evident, however, if we view Fig. 1 in the light of this sugar utilization, that the organisms growing in sugar media reached their limiting zones of volatile fatty acid production in the presence of sugar. This may have an important bearing on the growth curve of bacteria. This large yield of formic acid by these bacteria from glucose suggests a possible commercial problem.

Table I contains a summary of the determinations under the four sets of conditions. Fig. 1 pictures the results of the progressive series

[2] Franzen, H., and Egger, F., *J. prakt. Chem.*, 1911, lxxxiii, 323.

[3] Phelps, I. K., and Palmer, H. E., *J. Biol. Chem.*, 1917, xxix, 199.

studies and is included to furnish justification for choosing the 48 hour cultures for the main study.

TABLE I.

Volatile Fatty Acid Production of Dysentery Bacteria Under Different Conditions of Growth.

Organism.	Age of culture.	pH of culture.	Sugar present.	State.	Acid produced.			
					Formic. N/10	Acetic.	Propionic.	Butyric.
	days		*per cent*		*cc.*	*cc.*	*cc.*	*cc.*
D_1	2	4.9	1	Aerobic.	128.7	88.2	None.	None.
D_2	2	5.0	1	"	122.3	82.9	"	"
D_3	2	5.0	1	"	132.9	88.5	"	"
D_4	2	5.2	1	"	172.2	83.8	"	"
D_5	2	5.0	1	"	121.5	92.5	"	"
D_6	2	4.8	1	"	127.2	78.5	"	"
D_8	2	4.9	1	"	149.3	92.3	"	"
D_{10}	2	5.0	1	"	143.5	89.5	"	"
D_{11}	2	4.8	1	"	145.0	102.1	"	"
D_{13}	2	4.9	1	"	124.3	76.5	"	"
D_{17}	2	5.2	1	"	145.2	66.0	"	"
D_{23}	2	5.4	1	"	86.5	60.8	"	"
D_{24}	2	5.2	1	"	112.3	83.0	"	"
D_{33}	2	5.2	1	"	169.5	88.0	"	"
D_{37}	2	4.8	1	"	118.5	73.3	"	"
P Ty 16	2	5.1	1	"	77.3	80.2	"	"
Ty Round	2	5.1	1	"	150.5	78.6	"	"
D_1	10	7.5	None.	"	None.	33.2	12.8	"
D_2	10	7.6	"	"	"	35.2	13.3	"
D_4	10	7.5	"	"	"	27.9	11.1	"
D_{37}	12	7.4	"	"	"	31.5	11.8	"
D_1	12	6.2	"	Anaerobic.	31.2	27.1	None.	5.5
D_2	12	6.4	"	"	15.3	47.4	"	7.1
D_4	12	6.8	"	"	31.2	32.2	"	7.4
D_{37}	12	6.8	"	"	Determination lost.			
D_1	12	5.2	1	"	90.1	77.0	None.	None.
D_2*	14	5.2	1	"	87.5	56.5	"	"
D_4	12	5.5	1	"	116.4	78.2	"	"
D_{37}	12	5.4	1	"	94.8	68.5	"	"

* Growth less heavy to the eye.

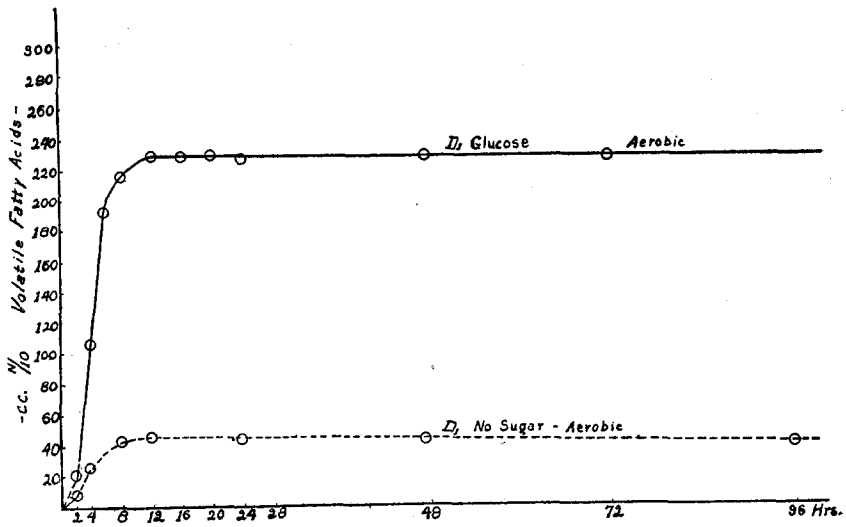

FIG. 1. Volatile fatty acid production curves of D_1 grown in 1,000 cc. of medium, and under the conditions indicated.

CONCLUSIONS.

These studies show:

1. A close agreement exists among all the organisms studied in the total quantity of volatile fatty acids produced and in the ratio of formic to acetic, under aerobic conditions, and in the presence of 1 per cent of glucose.

2. When grown upon peptone alone, with free access of air to the cultures, volatile fatty acids are produced in appreciable quantities, although the reaction of the solution has gone more alkaline as shown by colorimetric pH tests. Formic acid is not found, but in its place we obtain propionic acid.

3. Upon exhaustion of air from the non-sugar medium the bacteria again produce formic acid, and in addition some butyric. This is true for both Shiga and non-Shiga cultures. The reaction is distinctly more acid.

4. The presence of glucose in the medium from which the air has been pumped furnishes a condition which provokes about the same type and degree of fermentation that operates in the glucose medium bathed in air at atmospheric pressure.

5. The enormous quantity of formic acid produced by these bacteria may play a significant part in the digestive disturbances and toxic symptoms accompanying their infection of the human intestinal tract.