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Metabolism of Pathogenic Clostridia in Complex Carbohydrate-Rich Culture Media.*

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One of the characteristics of the pathogenic and many of the non-pathogenic clostridia is their marked ability to digest and further metabolize proteins and protein derivatives during growth in the usual culture media.[1-5] Ammonia, volatile acids, CO_2, and H_2 are produced during the anaerobic breakdown of proteins and amino acids.[1-7] Carbohydrates are the source of most of the energy in carbohydrate-rich media,† and large quantities may be consumed, due to the formation of neutral compounds, CO_2 and H_2. Dextrose yields ethyl alcohol and formic (CO_2 and H_2), acetic, butyric, and lactic acids.[2, 8-11] In the later stages of the fermentation the commercially important clostridia may yield very large quantities of acetone, and iso-propyl and butyl alcohols.[3, 9, 12] The same products may be obtained from lactic and pyruvic acids.[13, 14, 15] Acetic acid may be converted into acetone and butyl alcohol.[12] The presence of acetone and the higher alcohols in cultures of pathogenic clostridia

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4 Wolf, C. G. L., and Harris, J. E. G., *J. Path. Bact.*, 1918-1919, **22**, 1.

5 Wolf, C. G. L., *J. Path. Bact.*, 1918-1919, **22**, 115.

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† Wolf and Harris[4] believe that the volatile acids produced by *Cl. histolyticum* in cooked meat (without added sugar) medium are derived from glucosamine of the meat.

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14 Brown, R. W., Osburn, O. L., and Werkman, C. H., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 203.

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has not been reported. Acrolein is found in cultures of *Cl. perfringens (welchii)* in glycerol broth.[16] The presence of acrolein in fruit juices and the bitterness of wine have been attributed to the growth of this organism.[17] Acetone may be reduced to iso-propyl alcohol;[18] aldehydes and acids are reduced to alcohols;[2, 15, 18, 19, 20] and acetaldehyde may yield acetylmethyl carbinol.[20] The clostridia thus appear to be versatile in their abilities to carry out chemical reactions with a wide variety of substances.

A comparative quantitative study of the metabolism of the pathogenic clostridia under identical conditions of growth has never been made, nor have the principal products of metabolism in the usual liquid media with added dextrose been determined. In the present paper, data from 5 species of pathogenic clostridia are presented.

Experimental. The bacteriological and chemical methods were the same as described previously.[21] The inoculated media, con-

TABLE I.

The culture medium was prepared from fresh infusion of beef muscle (A), or meat extract (B), to which were added 1.0% of Difco Proteose-peptone (P), or Witte-peptone (W), 1.8% of Na_2HPO_4-12 H_2O, and 0.9% of dextrose. The initial reaction was pH 7.6.

Organism	Culture medium	Incubation 37° Hr	Dextrose consumed × 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.	Butyric acid mM per l.	Undetermined acidity cc N per l.	Yield lactic acid %
Cl. perfringens	A_5W	10	29.0	9.5	12.1	7.3	4.7	0.7	2.2	33
(welchii)	B_6P	24	57.4	30.2	19.0	9.0	9.4	1.2	—1.2	53
Cl. septicum	A_5W	10	22.5	1.3	13.5	6.3	3.9	2.7	0.7	6
(Vibrion septique)	B_6P	24	49.7	13.6	25.7	12.3	13.1	3.1	1.0	27
Cl. sporogenes	A_5W	10	17.4	0.3	9.5	7.2	5.0	0.8	—0.6	2
	B_6P	24	47.6	4.1	27.1	12.5	23.0	2.0	—2.4	9
" *tetani*	A_5W	10	11.9	—0.6	5.5	5.7	4.2	1.5	—3.9	loss
" *botulinum*, Type A	A_5W	10	14.4	—4.1	6.1	5.5	5.2	1.3	—1.4	loss
" " " "	B_6P	24	21.9	2.5	15.6	11.7	8.9	2.2	—6.9	11
" " " B	A_5W	10	19.5	0	14.9	10.2	6.5	1.0	—1.8	0
" " " "	B_6P	24	29.5	0.6	25.6	17.0	12.2	1.5	—6.4	2

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[18] Langlykke, A. F., and Fred, E. B., *J. Bact.*, 1937, **33**, 102.

[19] Blanchard, K. C., and MacDonald, J., *J. Biol. Chem.*, 1935, **110**, 145.

[20] Osburn, O. L., *Iowa State College J. Sci.*, 1935-1936, **10**, 97.

[21] Friedemann, T. E., *J. Bact.*, 1938, **35**, 527.

tained in tall, 4 oz dispensing bottles, were incubated from 10 to 24 hours at 37° to 38° in an atmosphere of pure nitrogen. Growth was stopped by the addition of exactly 1/10 volume of N H_2SO_4. Subcultures and smears were made just before acidification to determine the purity of the cultures. The organisms were obtained through the courtesy of Dr. G. M. Dack of the Department of Bacteriology.

The changes effected by growth of the organisms in two batches of culture media, differing somewhat in composition, are shown in Table I. The composition of the media is given at the top of the table.

Discussion of Results. The principal feature of the results shown in Table I is the large yield of formic acid, which, in most instances, tended to approach the sum of the mM of acetic acid and ethyl alcohol. *Cl. perfringens (welchii),* in culture medium A5W, consumed 14.5 mM of dextrose, or 29.0 mM of C_3, and yielded 12.1 mM of formic acid, and 12.0 mM of C_2-compounds. In the same batch of culture medium and during the same period of incubation *Escherichia coli* consumed an average of 26.9 mM of dextrose (or 53.8 mM of C_3) and yielded an average of 25.7 mM of formic acid, 15.4 mM of acetic acid, and 14.8 mM of ethyl alcohol. *Cl. perfringens* yielded 19.0 mM of formic acid and 18.4 mM of C_2-compounds in batch B6P. The results from *Cl. septicum (Vibrion septique)* were almost as regular. Slightly less formic acid than C_2-compounds was obtained from the other clostridia. No evolution of "gas" was seen when *Salmonella* and *Escherichia* were grown in batch A5W. On the other hand, "gas" was vigorously evolved in all cultures of the clostridia. This observation suggests that the clostridia may have decomposed other substances, besides formic acid, with the production of "gas".

Cl. perfringens, Cl. septicum, and *Cl. sporogenes* produced lactic acid. Their ability to yield lactic acid was in the order named. The yield from each organism was greatest in the medium in which the greatest consumption of sugar was found. Thus *Cl. perfringens* yielded 33% of lactic acid from 29.0 mM C_3; 53% was obtained from 57.4 mM C_3. *Cl. septicum* yielded 6% of lactic acid from 22.5 mM of C_3; and 27% from 49.7 mM C_3. *Cl. sporogenes* yielded 2% from 17.4 mM C_3, and only 9% from 47.6 mM C_3. The same trend is shown by the following data from *Escherichia coli* which was incubated at 37.5° in the same type of culture medium. The consumption of C_3 and the corresponding yield of lactic acid were as follows: 17.6 mM, 3%; 28.9 mM, 5%; 34.8 mM, 11%: 44.5 mM, 20%; 63 mM, 36%; 75.1 mM, 44%.

No conclusions can be reached from these data concerning the production of lactic acid by *Cl. tetani* and *Cl. botulinum,* since the consumption of sugar was small. These organisms removed lactic acid from the culture medium—a result so far not observed in this laboratory when intestinal organisms, streptococci, pneumococci, and staphylococci were grown anareobically over a short period of time.[21-25]

A striking feature of the metabolism of these clostridia is their ability to produce butyric acid. Measurable quantities of butyric acid have not so far been found by us in young cultures of other pathogenic bacteria.[21-25] Whether this acid was derived from sugar or from amino acids cannot, of course, be determined from the data. But it is significant that the quantity was small. The presence of butyl alcohol could not be demonstrated.‡

Our results differ greatly from those given in the literature. This is due, perhaps, to the long period of incubation, lasting from several days to several weeks, used by other workers. During the long incubation the organisms bring about many secondary changes, such as the decomposition of formic acid, the conversion of acetic acid to alcohol and other products, and the further metabolism of lactic acid. Our data indicate that the carbohydrate metabolism of *young, rapidly growing,* pathogenic clostridia is similar in many respects to that of many other rapidly growing pathogens.[21, 22, 24, 25]

A large part of the carbohydrate is converted either into lactic acid or it is further metabolized to yield approximately 2 molecules of formic acid, and 1 molecule each of acetic acid and ethyl alcohol.

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‡ The distribution coefficient of the volatile acids derived from the oxidation of the alcohols by chromic acid was the same as that of acetic acid.[19]