

METABOLISM OF B. WELCHII, VIBRION SEPTIQUE,  
B. FALLAX, B. TERTIUS, B. TETANI, B. PSEUDO-  
TETANI, B. BOTULINUS, B. BIFERMENTANS,  
B. OEDEMATIENS, B. AEROFOETIDUS,  
B. SPOROGENES, B. HISTOLYTICUS,  
AND B. PUTRIFICUS

STUDIES IN BACTERIAL METABOLISM, XLIV-LV

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BACILLUS WELCHII

STUDY XLIV

*Bacillus welchii*, also known as *Bacillus perfringens*, *Bacillus aerogenes capsulatus*, the "gas bacillus," and by several other names,<sup>1</sup> is an anaerobic bacillus, originally isolated and described by Welch and Nuttall<sup>2</sup> as a "gas-producing organism capable of rapid development in the body after death." Welch<sup>3</sup> has previously published a preliminary report on the bacillus, announcing its occurrence in the blood and emphysematous tissues of a patient who had died as the result of the rupture of an aortic aneurysm. About the same time Fraenkel<sup>4</sup> had described in rather general terms his *Bacillus phlegmones-emphysematosae*, and called attention to a probable causal relation of it to gas phlegmon (emphysematous-cellulitis or emphysematous-gangrene).

It seems probable that the Welch bacillus is the type member of either a group of closely related organisms, or of a series of identifiable variants of the same bacillus. A voluminous literature has grown up around the "gas bacillus," or Welch bacillus group, not only with reference to pathogenicity but also in regard to distribution in soil, dust, water, sewage and in the intestinal tract of man.<sup>1</sup> During the Great War it achieved conspicuous notoriety as the chief incitant of gas gangrene.<sup>5</sup> The resulting intense scrutiny to which the Welch bacillus

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<sup>1</sup> Simonds, Monograph 8, Rockefeller Institute, 1915.

<sup>2</sup> Johns Hopkins Hosp. Bull., 1892, 3, p. 81.

<sup>3</sup> Jour. Exper. Med., 1896, I, p. 5.

<sup>4</sup> Centralbl. f. Bakteriolog., 1893, 13, p. 13; Ueber Gas Phlegmon, 1893.

<sup>5</sup> Weinberg and Séguin: La Gangrène Gazeuse, 1917. Also, Report 39, Medical Research Committee, 1919.

has been subjected, both culturally, pathologically and serologically, has cleared up many of the extraordinary and extravagant claims of its "polymorphism" and other unusual departures from bacterial stability, and it is now apparently a well-defined entity whose salient characteristics are those described in the comprehensive monograph of Simonds.<sup>1</sup>

The biochemistry of this bacillus has received comparatively little attention, although it possesses some inherent features which are noteworthy, both with respect to the organism itself, and in contrast to or comparison with, closely related anaerobic bacteria. The observations on this aspect of the life history of the gas bacillus prior to the publication of Simonds' study are unreliable, because the earlier published descriptions convey almost conclusive evidence of contamination of the cultures studied with proteolytic anaerobic organisms, or even with aerobic microbes. Thus, Klein,<sup>6</sup> Tissier and Martelly,<sup>7</sup> and Grassberger and Schattenfroh,<sup>8</sup> described protein digestive powers, proteolytic enzymes, or even in the latter instance,<sup>9</sup> a "dimorphism," one phase of which was proteoclastic, to the Welch organism. Rosenthal<sup>10</sup> mentions an aerobic state, in which the morphology and characteristics of the organism change with increasing aerobic tolerance to the final acquisition of an oval, coccoid form, indistinguishable from that of *Micrococcus ovalis*.

The original studies of Welch and Nuttal<sup>2</sup> were quite clear with reference to evidence of proteolysis in gelatin. They state, "The bacillus is best classed among the non-liquefiers of gelatin, although in anaerobic cultures of 5 per cent. of sugar gelatin there may be a slight softening due to peptonization of the gelatin over a limited area, as is made manifest by the settling of the growth toward the bottom of the line of puncture in stab cultures, and by a slight displacement of the gas bacillus in changing the position of the tube. Cultures in gelatin which have developed at 35-37° C. become solid upon cooling the tube."

Simonds<sup>1</sup> was apparently the first observer actually to measure the chemical changes induced by Welch bacillus in mediums of known composition. His analytic figures show clearly that its action on protein (nutrient broth) is very small indeed. Carbohydrates (glucose, lactose, saccharose, starch and glycerol), on the contrary, are energetically attacked with the formation of much gas, considerable acid,

<sup>6</sup> Centralbl. f. Bakteriöl., 1895, 18, p. 737.

<sup>7</sup> Ann. Inst. Past., 1902, 16, p. 865.

<sup>8</sup> Arch. f. Hyg., 1902, 42, p. 219.

<sup>9</sup> Arch. f. Hyg., 1904, 48, p. 1.

<sup>10</sup> L'Aerobisation des microbes anaérobies, 1908. Compt. rend. Soc. de Biol., 1906, 58, p. 828.

and with minimal effect on the protein constituents of these fermentation mediums.

Wolf and Harris<sup>11</sup> carried out an elaborate series of metabolism studies on an anaerobic organism identified as *B. welchii*. A variety of mediums were used and observations during various periods of growth were made on the nitrogenous changes induced in these mediums, together with the rate and amount of gas formation. They found that the gas production by their organism in milk was quite variable, although in each instance a large volume was formed. Their actual figures of gas production per liter of medium at the end of approximately 24 hours varied from about 1,650 c.c. to as much as 3,820 c.c. This large liberation of gas is in accord with the most prominent feature of milk cultures of the gas bacillus, the so-called "stormy fermentation." The gas was found to be approximately two-thirds hydrogen, the remainder practically entirely carbon dioxide. These figures are in close accord with those of Theobald Smith.<sup>12</sup> The titratable acidity of the cultures rose from about 20 to 62 c.c. of normal acid per liter during the period of intense fermentative activity. The hydrogen-ion value in one experiment appears to have increased from  $P_H$  6.0 to approximately  $P_H$  4.58.

The nitrogenous changes observed by them were significant. In one experiment, which seems to be representative, the amino nitrogen increased from an initial content of 3.8 mg. per 100 c.c. of milk to 26.5 mg. in 100 c.c. of milk within an incubation period of 35 hours. The ammonia change during the same interval was very small. At the start the milk contained 4.0 mg. of ammonia per 100 c.c., and at the end of 33 hours' incubation the increase was only 1.6 mg. in the same volume. Somewhat more than 3 gm. of lactose per liter were changed to carbon dioxide and hydrogen, and organic acids, during this period.

The pressure generated by the rapid evolution of gas in the milk was very considerable. In one experiment the culture generated gas to the extent of 1.5 times the entire initial volume of the medium, and the pressure in a mercury manometer rose to the astonishing height of 1,520 mm. of mercury—about 2 atmospheres. It will be remembered that Taylor<sup>13</sup> has previously shown that a pressure of gas exceeding 1.5 atmosphere was generated in one of his cultures of the gas bacillus within 5 hours, and the exact pressure was unrecorded because the

<sup>11</sup> Jour. Path. & Bacteriol., 1917, 21, p. 386.

<sup>12</sup> Ztschr. f. Infektionskrankh. d. Haustiere, 1906, I, p. 26. Jour. Med. Res., 1905, 14, p. 193.

<sup>13</sup> Lancet, 1916, I, p. 123.

manometer became disarranged at this time. Taylor believed that the pressure exerted in muscular tissues by the generation of gas might be a potent factor both in spreading the infection of the Welch bacillus through the tissues by forcing apart the muscle fibers, and by actually causing serious mechanical damage to the muscular tissues through intramuscular gas pressure.

Wolf and Harris<sup>11</sup> also performed metabolism experiments in sugar-free broth, using a tryptic digest of casein carefully freed from sugar as a medium. It was found that about 20 mg. of amino-nitrogen and 42.5 mg. of ammonia were formed per 100 c.c. of medium in 27 hours. The reaction underwent very little change, from  $P_H$  7.68 to  $P_H$  7.49. Wolf and Harris emphasize the rise in amino nitrogen in their cultures, in contrast to the negligible amount of ammonia produced simultaneously. The reverse appears to have been observed in cultures of *Vibrio septique*.<sup>14</sup> They state that an odor suggestive of cultures of *B. sporogenes* was detected at the end of the experiment, but were apparently unable to detect a contaminant, if such existed. They very properly state that the results are not suggestive of contamination with *B. sporogenes*, which would undoubtedly have formed much more ammonia than that found in this experiment.

A study of the analytic figures of Wolf and Harris reveals clearly the sparing action which utilizable carbohydrate exerts for protein in cultures of the organism containing both sugar and protein available as sources of energy. These studies emphasize particularly the rapidity of growth of the organism they identified as the Welch bacillus and indicate clearly that the bacillus is carbohydrophilic rather than proteophilic. Action on protein and protein derivatives, in fact, was found to be minimal, even in the absence of utilizable carbohydrate, which is wholly in accord with the observations of Simonds.<sup>1</sup>

A peculiar and striking feature of the growth of the Welch bacillus is the formation of a soluble poison, reminiscent in many respects of the soluble toxins of organisms like *B. diphtheriae*. Bull and Pritchett<sup>15</sup> discovered that filtrates of 18-24 hour cultures of *B. welchii*<sup>16</sup> contain a soluble poison which incites many of the signs and symptoms of gas gangrene in experimental animals. A striking peculiarity of this poisonous substance is its diminution in potency as the culture becomes older. Indeed, after 48 hours' incubation the

<sup>14</sup> Jour. Path. & Bacteriol., 1918, 22, p. 115.

<sup>15</sup> Jour. Exper. Med., 1917, 26, p. 119.

<sup>16</sup> Culture 617 D, of Simonds.

poisonous properties of the filtrate may be practically negligible, even though 18-24 hour samples of the same culture, freed from organisms, were powerfully toxic. The presence of utilizable sugars has little or no apparent influence in reducing the production of this poison, contrasting in this respect sharply with the formation of soluble toxin in cultures of the diphtheria bacillus.<sup>17</sup> It will be remembered that diphtheria toxin is formed relatively slowly in protein mediums, reaching the maximum about the eighth or tenth day of incubation; also the presence of more than small amounts of utilizable carbohydrate prevents the formation of soluble toxin in cultures of the diphtheria bacillus. The genesis of the gas bacillus poison, therefore, differs from the formation of the soluble toxin of the diphtheria bacillus in at least two particulars. First, it appears early in the life history of the culture when the multiplication of the organisms—the birth rate, in other words—is maximal, and before the accumulation of products indicative of the energy phase of large numbers of mature bacilli becomes manifest. Secondly, the soluble poison of the Welch bacillus decreases rapidly in potency during the period when the energy phase of the life history of the culture has become dominant, and has succeeded the initial phase of rapid multiplication. It may be surmised that the soluble poison of the gas bacillus, therefore, is a substance or substances produced incidentally during the reproductive process of the organism. It is a waste product of structure rather than of energy. The rapid diminution in potency may be due to lability of the poison, or to its actual neutralization, disintegration or destruction, as the energy phase of the culture with its resulting products becomes prominent. Filtrates of cultures at the height of poison production, freed from bacteria, appear to be relatively stable, however, again suggesting that the phenomena attending the energy phase of the culture are destructive of the poisonous substance.

The gas bacillus poison, injected into suitable animals in properly spaced and measured amounts, incites the formation of specific antibodies, or at least of substances capable of neutralizing this poisonous substance. In this respect, the soluble, poisonous substance is analogous to the known soluble toxins in that both incite specific reactions in suitable hosts.

The experiments on the metabolism of the Welch bacillus, recorded in the following, and those in succeeding contributions, were under-

<sup>17</sup> Theobald Smith, *Jour. Exper. Med.*, 1899, 4, p. 373.

taken on a request from the Medical Section of the National Research Council that a study be made of the biochemistry of the anaerobic bacteria which are associated with wounds of warfare. This work has been pursued for more than four years. A part of this time, however, was spent in searching for methods of isolation and culture of anaerobic organisms of greater precision than those available at the beginning of the investigation. The greatest problem encountered was that of securing cultures of undoubted purity. Isolation of single spores has been found extremely tedious, but reliable; hence, all cultures of the anaerobic bacteria studied were purified thrice by the modified Barber single cell method, previously described.<sup>18</sup> The cultures intended for metabolic study were grown in specifically designed flasks, each holding about 110 c.c. of medium. An individual flask was inoculated for each kind of medium for each day of observation. A set of 60 flasks, therefore, was required for each organism, including of course suitable controls. It is believed that this procedure of single flasks for each medium each day far exceeds in accuracy the alternate plan of removing samples at stated intervals from the same flask. The smoothness of the "growth curves" charted from the analyses is indicative of the precision of the entire process.

The several kinds of mediums (shown in the analytic tables) were seeded at the same time with two drops of an active meat-liver medium culture of the organism under consideration. Incubation was practiced at 37 C. for the various times indicated in the tables.

The analytic methods employed were those described in previous communications.<sup>19</sup> The determinations included the change in titratable acidity,<sup>20</sup> the measurement of changes in the nitrogenous constituents of the mediums—ammonia formation, amino acid liberation and utilization—the total nitrogen content, and attempts to demonstrate soluble proteolytic enzymes. In other words, the nitrogenous metabolism of the culture was followed, precisely as the nitrogenous metabolism of the body is followed through the measurement of the nitrogenous constituents of the blood and the urine.

Previous studies on the metabolism of aerobic and facultatively anaerobic organisms<sup>19, 21</sup> have shown material differences between the

<sup>18</sup> Kendall, Ryan and Cook: *Jour. Infect. Dis.*, 1921, 29, p. 227.

<sup>19</sup> Kendall and Farmer: *Jour. Biol. Chem.*, 1912, 12, p. 13. Kendall, Day and Walker: *Jour. Am. Chem. Soc.*, 1913, 35, p. 1201. Kendall and Walker: *Jour. Infect. Dis.*, 1915, 17, p. 442.

<sup>20</sup> These studies were started before the indicator method for hydrogen-ion measurement was developed to a satisfactory degree.

<sup>21</sup> Sears: *Jour. Infect. Dis.*, 1916, 19, p. 105.

TABLE 1  
BACILLUS WELCHII, 617D

Day	Plain			Gelatin			Glucose			Mannitol			Glycerol			Lactose			Saccharose			Starch			Milk		
	Reaction	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen
Control April, 1917	-0.60	4.2 21.0	2.14	-0.90	4.2 43.4	0.47	-0.20	4.2 21.0	2.14	-0.70	3.5 23.1	1.77	-0.70	3.5 23.1	1.77	-0.50	4.2 21.0	2.14	-0.60	4.2 22.4	2.14	-0.70	2.8 22.4	1.42	-0.70	4.2 22.4	1.42
1	-0.60	12.6 35.7	6.42	+0.10	11.9 46.9	1.35	+2.90	4.9 25.9	2.50	-0.80	10.5 33.1	5.36	+1.10	11.2 26.6	5.70	+2.40	5.6 23.4	2.86	+2.50	7.0 28.0	3.58	+2.00	10.5 31.5	5.36	+2.00	10.5 31.5	5.36
3	-0.70	16.8 40.6	8.58	+0.10	16.8 49.0	1.91	+2.90	4.9 25.9	2.50	-0.80	15.4 43.4	7.36	+1.50	11.2 26.6	5.70	+2.40	5.6 23.4	2.86	+2.50	7.0 28.0	3.58	+2.00	11.2 30.8	5.70	+2.00	11.2 30.8	5.70
6	-0.70	18.2 50.4	9.30	+0.10	18.2 56.0	2.05	+2.70	4.9 23.8	2.50	-0.90	16.8 51.8	8.58	.....	.....	.....	+2.20	5.6 25.0	2.86	+2.50	7.0 28.0	3.58	+2.00	11.2 30.8	5.70	+2.00	11.2 30.8	5.70
13	-0.70	19.6 52.5	10.00	+0.10	23.8 78.4	2.70	+2.30	5.6 25.2	2.85	-1.00	18.2 56.3	9.40	+1.50	11.2 26.6	5.70	+2.20	5.6 25.0	2.86	+2.00	7.7 28.7	3.93	+1.90	11.9 31.5	6.01	+1.30	8.4 14.7	3.19
Control April, 1918	-0.40	4.9 23.8	2.46	-0.70	5.6 46.2	0.59	-0.40	4.9 23.8	2.46	-0.50	5.6 23.8	2.95	-0.60	4.9 22.4	2.40	-0.40	5.6 23.8	2.95	-0.40	5.6 22.4	2.95	-0.55	5.6 23.1	2.95	+1.10	5.6 14.7	2.96
1	-0.40	12.6 36.6	6.64	-0.30	10.5 50.4	1.10	-2.20	4.2 21.7	2.22	-0.70	12.6 24.5	6.64	+1.90	10.5 25.9	5.55	+2.20	5.6 25.2	2.95	+2.50	6.3 22.4	3.33	+3.20	11.9 28.7	6.30	+3.60	8.4 9.1	1.97
3	-0.50	16.8 50.4	8.90	0.00	13.3 52.5	1.39	-2.20	4.9 23.8	2.46	-0.70	16.1 39.9	8.90	+1.90	11.9 25.9	6.30	+2.40	15.4 43.4	8.16	+2.80	8.4 30.1	4.44	+3.00	11.9 28.7	6.30	+4.00	9.1 11.9	2.40
6	-0.70	17.5 58.8	9.28	+0.10	15.4 53.9	1.62	+2.20	5.6 25.2	2.95	-0.80	16.8 48.4	8.90	+2.00	10.5 25.2	5.55	+2.30	5.6 30.1	2.95	+2.70	7.7 30.1	4.07	+2.90	11.9 28.7	6.30	+3.80	9.1 11.9	2.40
14	-0.70	20.3 58.1	10.06	+0.15	20.3 65.1	2.13	+2.00	5.6 25.2	2.95	-0.80	19.6 57.4	10.08	+2.00	11.9 28.7	6.30	+2.00	7.7 30.1	4.07	+2.00	8.4 30.1	4.41	+2.30	12.6 30.8	6.64	+4.00	11.2 16.1	3.24
Control March, 1920	-0.60	17.5 20.3	7.81	-0.70	4.2 36.4	0.62	-0.60	13.9 21.7	8.43	-0.70	18.2 21.0	8.13	-0.60	17.5 21.7	7.81	-0.60	18.2 21.0	8.12	-0.60	18.2 21.0	.....	-0.50	17.5 21.7	.....	+1.70	4.2 25.2	5.46
1	-0.80	25.9 30.1	11.55	-0.80	3.5 39.2	0.52	+4.70	21.0 22.2	9.38	-0.80	27.3 31.5	12.30	+3.00	23.1 28.0	10.08	+3.40	23.1 25.7	12.80	+4.40	25.9 30.8	11.55	+4.30	24.5 34.3	10.90	+3.50	4.2 9.1	2.51
3	-0.70	28.7 49.7	12.81	-0.50	11.9 42.0	1.77	+4.80	21.7 32.9	9.70	-0.80	32.2 37.8	14.30	+3.20	25.9 30.8	11.20	+3.40	25.2 29.4	11.20	+4.40	25.9 30.8	11.55	+4.60	25.9 34.4	11.55	+3.60	6.3 12.6	2.73
6	-0.70	28.7 49.7	12.81	-0.40	11.2 46.9	1.67	+4.90	23.1 35.0	10.03	-0.80	35.0 42.7	15.70	+4.00	24.5 30.1	10.90	+3.60	25.9 32.9	11.55	+4.40	25.9 32.9	11.55	+4.90	26.6 35.0	11.80	+3.60	7.0 13.3	2.88
9	-0.80	34.3 53.9	15.23	-0.60	3.5 56.7	0.52	+5.00	22.4 35.0	10.00	-0.80	35.0 42.7	15.70	+3.80	24.5 30.1	10.90	+3.60	25.9 35.7	11.55	+4.50	26.6 32.9	11.80	+5.10	26.6 36.4	11.80	+4.00	7.7 16.8	3.64
14	-0.90	36.4 58.1	16.24	+0.20	21.0 56.7	3.13	+5.10	22.4 35.7	10.00	-0.90	34.3 53.9	15.30	+3.50	24.5 30.1	10.90	+3.40	25.2 35.7	11.20	+4.60	26.2 33.6	11.20	+5.10	27.3 37.1	12.20	+4.00	7.7 18.2	3.94

Reaction: --- = alkaline to neutral red; + = acid to neutral red; cc normal acid or alkali per 100 cc of medium.

Ammonia and amino nitrogen expressed in milligrams per 100 cc of culture medium.

Control indicates initial composition of medium. Net results may be obtained by subtracting the control from the day's analysis.

Amino nitrogen is corrected in each instance for ammonia.



TABLE 2  
BACILLUS WELCHII

Day		Platin			Gelatin			Glucose			Mannitol			Glycerol			Lactose			Saccharose			Starch			Milk		
		Reaction	Amino Nitrogen	Percentage Ammonia	Reaction	Amino Nitrogen	Percentage Ammonia	Reaction	Amino Nitrogen	Percentage Ammonia	Reaction	Amino Nitrogen	Percentage Ammonia	Reaction	Amino Nitrogen	Percentage Ammonia	Reaction	Amino Nitrogen	Percentage Ammonia	Reaction	Amino Nitrogen	Percentage Ammonia	Reaction	Amino Nitrogen	Percentage Ammonia	Reaction	Amino Nitrogen	Percentage Ammonia
Control	1	-0.50	3.523.1	2.00	-0.50	4.242.0	0.47	-0.20	3.520.3	2.00	-0.50	3.523.1	2.00	-0.60	3.523.1	2.00	-0.60	3.523.1	2.00	-0.60	3.523.1	2.00	-0.60	3.523.1	2.00	-0.60	3.523.1	2.00
	3	-0.60	12.623.8	7.20	-0.60	4.542.0	0.75	-2.60	4.221.0	2.40	-0.60	11.927.8	6.80	-1.80	10.523.1	6.00	-3.30	4.227.3	2.80	-2.30	4.925.2	2.80	-2.80	10.531.5	6.00	-2.80	10.531.5	6.00
	8	-0.60	14.723.8	8.40	-0.60	7.549.0	0.75	-2.60	4.221.0	2.40	-0.60	14.033.6	8.00	-1.60	10.523.1	6.00	-3.40	4.927.3	2.80	-2.30	4.925.2	2.80	-2.80	10.531.5	6.00	-2.80	10.531.5	6.00
	13	-0.50	16.823.0	9.51	-0.30	9.822.5	1.11	-2.50	4.221.0	2.40	-0.50	16.441.8	8.80	-1.70	10.523.1	6.00	-3.00	5.625.0	3.20	-2.10	5.625.2	3.20	-2.60	11.232.2	6.40	-2.60	11.232.2	6.40
	20	-0.50	18.235.0	10.40	-0.40	14.163.7	1.64	-2.70	4.221.0	2.40	-0.50	16.846.9	9.00	-1.30	11.226.6	6.00	-1.80	5.630.1	3.20	-1.80	4.928.7	2.80	-2.50	11.932.2	6.80	-2.50	11.932.2	6.80
Control	1	-0.50	3.521.7	1.92	-0.90	2.542.0	0.33	-0.20	2.820.3	1.54	-0.60	2.822.4	1.54	-0.50	2.822.4	1.54	-0.50	2.822.4	1.54	-0.40	2.822.4	1.54	-0.40	2.822.4	1.54	-0.40	2.822.4	1.54
	3	-0.60	9.120.8	5.00	-0.80	4.246.9	0.49	-2.40	3.521.7	1.92	-0.40	9.822.4	5.33	-1.40	7.023.1	3.88	-3.80	1.426.6	0.77	-2.80	1.428.0	0.77	-2.60	9.126.6	5.00	-2.60	9.126.6	5.00
	8	-0.60	10.535.7	6.58	-0.60	7.549.0	0.91	-2.40	4.221.7	2.31	-0.60	11.228.0	6.16	-1.70	7.023.1	4.23	-2.60	2.826.6	1.54	-2.40	2.132.2	1.15	-2.60	9.126.6	5.00	-2.60	9.126.6	5.00
	13	-0.50	11.931.7	6.84	-0.60	11.656.7	1.35	-2.20	4.923.1	2.69	-0.60	13.808.8	7.39	-1.60	7.023.9	4.23	-2.00	3.528.7	1.92	-2.10	2.139.2	1.15	-2.60	9.126.6	5.00	-2.60	9.126.6	5.00
Control	1	-0.50	16.135.7	8.81	-0.80	16.461.6	1.80	-2.20	4.925.9	2.69	-0.50	16.135.7	8.84	-1.50	8.428.0	4.61	-2.00	4.228.7	2.82	-2.00	2.842.7	1.54	-2.60	9.829.4	5.38	-2.60	9.829.4	5.38
	3	-0.60	4.221.0	2.14	-0.90	3.543.4	0.39	-0.20	4.221.0	2.14	-0.70	3.523.1	1.79	-0.70	3.523.1	1.79	-0.50	4.221.0	2.14	-0.60	4.222.4	2.14	-0.70	2.822.4	1.42	-0.70	2.822.4	1.42
	8	-0.50	4.220.1	2.14	-0.80	8.449.7	0.95	-2.80	4.924.5	2.50	-0.60	2.830.1	1.43	-0.70	7.725.2	3.92	-3.00	4.930.1	2.50	-2.80	4.229.4	2.14	-2.20	8.430.1	4.28	-2.20	8.430.1	4.28
	13	-0.60	12.642.7	6.43	-0.80	12.644.6	1.43	-2.60	4.925.9	2.50	-0.80	9.832.9	5.00	-0.70	8.425.9	4.28	-2.80	4.930.1	2.50	-3.00	4.229.4	2.14	-2.20	9.829.4	5.00	-2.20	9.829.4	5.00
Control	1	-0.50	13.345.3	6.78	-0.80	14.759.5	1.67	-2.50	5.628.0	2.86	-0.90	11.241.3	5.72	-0.60	8.425.9	4.28	-2.70	5.642.2	2.80	-2.70	5.642.2	2.80	-2.00	8.433.6	4.28	-2.00	8.433.6	4.28
	3	-0.50	3.523.1	2.00	-0.80	16.139.5	1.83	-2.50	5.628.0	2.86	-0.80	14.756.7	7.50	-0.60	8.425.9	4.28	-2.10	5.632.2	2.86	-2.20	5.632.2	2.86	-2.00	8.433.6	4.28	-2.00	8.433.6	4.28
	8	-0.50	4.240.6	0.47	-0.90	4.240.6	0.47	-0.10	3.520.3	2.00	-0.60	3.521.7	2.00	-0.50	2.822.4	1.60	-0.50	3.521.7	2.00	-0.50	3.521.7	2.00	-0.50	2.822.4	1.60	-0.50	2.822.4	1.60
	13	-0.50	7.025.9	4.00	-0.80	7.040.6	0.78	-2.40	4.923.1	2.80	-0.50	7.026.6	4.00	-0.50	7.023.8	4.00	-2.40	4.925.0	3.20	-2.60	4.227.3	2.40	-2.80	7.730.1	4.40	-2.80	7.730.1	4.40
Control	1	-0.50	10.534.8	6.00	-0.80	9.144.8	1.02	-2.40	4.923.1	2.80	-0.60	8.431.5	4.80	-1.00	7.025.9	4.00	-3.00	5.628.4	3.20	-2.60	4.227.3	2.40	-3.00	7.730.1	4.40	-3.00	7.730.1	4.40
	3	-0.60	10.539.2	6.00	-0.80	11.956.0	1.38	-2.20	4.923.1	2.80	-0.60	9.834.9	5.80	-0.70	8.428.0	4.80	-2.80	5.642.2	3.20	-2.60	4.227.3	2.40	-2.80	7.730.1	4.40	-2.80	7.730.1	4.40
	8	-0.60	13.339.2	7.60	-0.60	14.756.0	1.72	-2.20	4.923.1	2.80	-0.60	14.039.9	8.00	-0.60	8.426.6	4.80	-1.60	5.630.8	3.20	-2.00	4.929.4	2.80	-2.20	7.730.1	4.40	-2.20	7.730.1	4.40
	13	-0.50	3.523.1	2.00	-0.90	4.240.6	0.47	-0.10	3.520.3	2.00	-0.60	3.521.7	2.00	-0.50	2.822.4	1.60	-0.50	3.521.7	2.00	-0.50	3.521.7	2.00	-0.50	2.822.4	1.60	-0.50	2.822.4	1.60
Control	1	-0.50	7.026.6	4.00	-0.80	6.343.4	0.99	-2.30	4.222.4	2.40	-0.50	6.327.8	3.60	-0.60	7.025.9	4.0	-1.80	4.224.5	2.40	-2.30	4.225.2	2.40	-2.20	7.026.6	4.00	-2.20	7.026.6	4.00
	3	-0.60	10.537.1	6.00	-0.80	14.048.3	1.56	-2.20	4.222.4	2.40	-0.60	10.537.1	6.00	-1.00	7.026.6	4.0	-1.90	4.226.6	2.40	-2.00	4.225.9	2.40	-2.10	7.026.6	4.00	-2.10	7.026.6	4.00
	8	-0.60	10.537.1	6.00	-0.70	14.038.2	1.66	-2.40	4.222.4	2.40	-0.60	7.729.4	4.10	-0.90	7.026.6	4.0	-1.80	4.226.6	2.40	-2.00	4.225.9	2.40	-2.20	7.026.6	4.00	-2.20	7.026.6	4.00
	13	-0.60	13.339.2	7.61	-0.70	19.656.7	2.19	-2.20	4.222.4	2.40	-0.60	13.342.0	7.61	-0.80	7.728.0	4.40	-1.70	4.228.0	2.40	-1.90	4.225.2	2.40	-2.20	8.430.1	4.80	-2.20	8.430.1	4.80



Control	..	-0.60	3.5/23.1	1.79	-0.50	3.5/39.9	0.40	-0.20	3.5/18.9	1.79	-0.60	3.5/21.7	1.79	-0.40	2.8/22.4	1.44	-0.50	3.5/21.7	1.79	-0.60	3.5/21.7	1.79	-0.60	3.5/21.7	1.44	+1.20	4.9/22.4	1.06
B. welchii "F"	1	-0.70	9.1/32.2	4.64	-0.60	3.5/41.3	0.40	-0.20	4.9/25.2	2.15	-0.70	8.4/28.0	4.93	+0.50	4.9/22.4	2.14	+1.30	5.6/28.0	2.86	+2.70	5.6/30.8	2.86	+1.10	9.1/38.4	4.64	+3.20	7.7/13.3	1.66
	3	-0.70	10.5/37.7	5.36	-0.60	7.0/49.0	0.80	-0.20	4.9/25.2	2.15	-0.70	10.5/38.0	5.36	+0.50	4.9/25.2	2.50	+1.60	5.6/28.0	2.86	+2.70	5.6/30.8	2.86	+1.10	9.5/37.8	5.00	+2.70	8.4/12.6	1.62
	6	-0.80	11.2/39.2	5.71	-0.60	9.8/53.7	1.10	-0.20	4.9/25.2	2.15	-0.70	10.5/39.0	5.36	+0.50	4.9/24.5	2.50	+1.60	5.6/28.0	2.86	+2.70	5.6/30.8	2.86	+2.30	10.5/37.1	5.36	+3.60	9.8/20.3	2.12
	13	-0.80	14.0/49.7	7.14	-0.90	13.3/58.8	1.49	-0.20	4.9/25.2	2.15	-0.80	12.6/48.2	4.93	+0.50	4.9/25.2	2.50	+1.70	7.0/30.4	3.60	+2.00	7.0/30.4	3.60	+2.10	13.3/59.9	6.86	+3.60	11.9/21.7	2.37
	20	-0.70	14.7/49.7	7.50	-0.10	14.7/65.1	1.66	-0.20	4.9/25.9	2.51	-0.70	10.5/41.3	5.36	+0.50	4.9/28.0	2.50	+1.80	7.0/30.4	3.60	+2.00	7.0/30.4	3.60	+2.10	14.7/42.7	7.50	+3.60	12.6/29.0	2.33
Control	..	-0.60	4.2/21.0	2.14	-0.90	14.2/43.4	0.48	-0.20	4.2/21.0	2.14	-0.70	3.5/23.1	1.79	-0.70	3.5/23.1	1.79	-0.50	4.2/21.0	2.14	-0.60	4.2/22.4	2.14	-0.70	2.8/22.4	1.43	+1.40	4.2/22.4	0.91
B. welchii "G"	1	-0.80	8.4/32.2	4.28	-1.10	8.4/42.0	0.95	-0.20	5.6/32.4	2.06	-0.90	8.4/30.8	4.93	+0.70	7.7/25.9	3.93	+2.50	6.3/30.1	3.93	+3.10	7.0/20.5	3.57	+1.20	9.8/26.6	5.00	+3.60	7.0/21.7	1.52
	3	-0.80	11.9/46.9	6.08	-1.20	10.5/51.1	1.19	-0.20	5.6/34.5	2.06	-0.90	10.5/38.6	4.93	+0.70	7.7/25.9	4.04	+2.50	6.3/30.1	3.93	+3.10	6.3/28.7	3.22	+2.30	11.9/50.2	6.08	+3.60	7.7/23.7	1.67
	6	-0.90	11.9/52.5	6.08	-1.30	10.5/53.7	1.19	-0.20	5.6/35.9	2.06	-1.00	11.2/49.6	6.72	+0.60	10.1/32.3	4.54	+2.50	7.7/32.2	3.93	+3.10	6.3/28.7	3.22	+3.90	11.9/50.2	6.08	+3.60	8.4/37.1	1.84
	13	-0.80	11.9/52.5	6.08	-1.30	15.4/68.5	1.75	-0.20	5.6/35.2	2.93	-1.00	11.1/59.2	6.08	+0.50	9.8/37.8	5.00	+2.40	7.7/32.2	3.93	+3.10	6.3/28.7	3.22	+3.20	11.9/50.2	6.08	+3.70	7.7/37.1	1.67
Control	..	-0.60	4.2/21.0	2.14	-0.90	4.2/43.4	0.48	-0.20	4.2/21.0	2.14	-0.70	3.5/23.1	1.79	-0.70	3.5/23.1	1.78	-0.50	4.2/21.0	2.14	-0.60	4.2/22.4	2.14	-0.70	2.8/22.4	1.43	+1.40	4.2/22.4	0.91
B. welchii "H"	1	-0.80	8.4/32.0	4.28	-1.00	8.4/42.0	0.95	-0.20	5.6/32.4	2.06	-0.90	8.4/24.5	4.78	+1.00	7.7/23.8	3.93	+2.50	7.0/33.6	3.57	+3.70	4.2/30.8	2.16	+1.20	9.8/26.6	4.99	+1.60	7.0/31.5	1.52
	3	-0.80	11.2/40.6	5.72	-1.00	10.5/51.1	1.19	-0.20	5.6/33.8	2.06	-0.90	10.5/49.3	4.69	+0.90	7.7/25.2	3.93	+2.50	7.0/33.6	3.57	+3.70	4.2/30.8	2.16	+2.90	10.5/39.9	5.36	+1.60	7.0/31.5	1.67
	6	-0.80	11.3/49.7	6.08	-0.90	10.5/56.7	1.19	-0.20	5.6/33.8	2.06	-0.90	10.5/49.3	4.69	+0.90	7.7/25.2	3.93	+2.50	7.0/33.6	3.57	+3.70	4.2/30.8	2.16	+3.90	11.3/55.7	6.07	+2.10	8.4/33.6	1.82
	13	-0.70	13.3/58.1	6.79	-0.80	15.4/68.5	1.74	-0.20	5.6/33.8	2.86	-1.00	13.3/58.0	6.74	+1.10	7.7/28.7	3.93	+2.60	7.0/35.0	3.57	+3.10	6.3/33.1	3.23	+3.20	11.9/55.7	6.07	+2.30	8.4/33.6	1.82
Control	..	-0.60	17.5/20.3	7.81	-0.70	4.2/36.4	0.15	-0.60	19.6/21.7	8.74	-0.70	18.2/21.0	8.12	-0.60	18.9/21.7	8.46	-0.60	18.9/21.0	8.46	-0.60	18.2/21.0	8.12	-0.50	18.2/21.7	8.12	+1.30	3.5/20.3	.....
B. welchii "I"	1	-0.90	93.7/99.4	19.80	-0.50	5.6/36.4	0.21	+4.00	24.5/30.8	10.90	-0.90	90.1/95.9	13.40	+1.40	28.7/26.6	12.70	+3.50	24.5/28.0	10.90	+4.30	22.4/26.6	10.00	+5.30	28.7/31.5	12.80	+2.30	6.3/19.6	1.36
	3	-0.80	93.3/91.5	15.30	-1.00	5.6/36.4	0.21	+4.00	25.2/30.8	11.25	-0.90	93.6/97.8	13.00	+1.60	30.8/26.6	13.75	+3.80	24.5/28.0	10.90	+4.20	24.5/30.1	10.00	+5.50	28.0/31.5	12.50	+2.80	9.1/37.8	1.97
	6	-0.80	93.6/94.4	14.30	-0.80	16.1/41.3	1.92	+4.20	25.2/35.7	11.25	-1.00	93.6/97.8	13.00	+2.30	35.3/30.8	14.35	+4.00	25.0/35.0	12.50	+4.50	25.9/35.9	11.55	+5.50	28.7/31.5	12.80	+3.30	9.1/39.2	1.97
	9	-0.80	93.0/41.3	13.68	-0.80	13.3/47.6	1.50	+4.30	25.9/32.9	11.55	-1.00	96.4/46.5	16.20	+2.00	31.5/31.5	14.35	+3.80	25.9/35.7	11.57	+4.60	25.9/35.9	11.55	+5.10	28.7/35.0	12.50	+3.20	9.1/39.2	1.97
Control	..	-0.50	3.5/23.1	1.85	-0.80	4.9/44.1	0.54	-0.20	3.5/21.7	1.85	-0.40	3.5/23.1	1.85	-0.40	3.5/23.1	1.85	-0.40	3.5/23.1	1.85	-0.50	3.5/23.1	1.85	-0.40	3.5/23.1	1.85	+1.40	7.7/23.1	1.47
B. welchii "J"	1	-0.60	9.8/32.9	5.18	-0.60	4.9/47.6	0.54	-0.20	4.9/35.2	2.96	-0.60	10.5/30.1	5.55	+1.60	7.0/27.3	3.70	+2.60	5.6/30.8	2.96	+2.60	4.9/28.7	2.59	+2.40	7.7/29.4	4.07	+2.20	9.1/21.0	1.87
	3	-0.60	10.5/39.2	5.55	-0.70	9.1/51.8	1.01	-0.20	4.9/35.9	2.59	-0.60	10.5/35.7	5.55	+1.70	7.0/27.3	3.70	+2.60	5.6/30.8	2.96	+2.70	4.9/28.7	2.59	+2.80	7.7/29.4	4.07	+2.20	8.4/31.5	1.60
	6	-0.70	11.2/46.7	5.94	-0.60	12.6/60.2	1.39	-0.20	4.9/35.9	2.59	-0.60	10.5/47.6	5.55	+1.40	7.0/27.3	3.70	+2.60	5.6/30.8	2.96	+2.70	4.9/28.7	2.59	+2.80	7.7/29.4	4.07	+2.20	9.8/37.1	1.87
	13	-0.70	14.7/58.8	7.78	-0.70	16.1/77.0	1.79	-0.20	4.9/35.9	2.59	-0.70	14.0/47.6	7.41	+1.20	7.7/28.0	4.07	+2.30	5.4/34.3	4.44	+2.00	4.9/28.7	2.59	+2.70	7.7/30.8	4.07	+2.30	14.7/58.2	2.80
	21	-0.70	14.0/58.1	7.41	-0.70	16.1/77.7	1.79	-0.20	7.7/80.1	4.07	.....	.....	.....	.....	7.7/28.0	4.07	+2.30	7.7/32.9	4.07	+2.00	5.6/30.1	2.96	.....	.....	.....	+3.00	14.7/58.2	2.80
Control	..	-0.50	3.5/23.1	1.98	-0.90	4.2/40.6	0.47	+0.10	3.5/30.3	1.98	-0.60	3.5/21.7	1.98	-0.50	2.8/22.4	1.60	-0.50	3.5/21.7	1.98	-0.50	3.5/21.7	1.98	-0.50	2.8/22.4	1.60	+1.40	4.2/21.0	0.86
B. welchii "K"	1	-0.50	7.7/29.4	4.40	-0.80	4.2/42.0	0.47	-0.20	4.9/32.1	2.78	-0.80	7.7/28.0	4.40	+1.00	7.7/25.9	4.40	+3.20	7.0/31.5	4.00	+2.60	7.0/28.7	4.00	+2.80	7.7/30.8	4.40	+3.60	7.0/18.2	1.43
	3	-0.80	12.6/32.2	6.43	-0.70	6.3/44.1	0.70	-0.20	4.9/32.1	2.78	-0.80	10.5/37.8	6.00	+1.10	7.7/25.9	4.40	+3.20	7.0/30.8	4.00	+2.70	7.0/28.7	4.00	+2.80	8.4/30.8	4.40	+4.00	6.3/23.1	1.29
	6	-0.70	12.6/44.1	7.20	-0.90	11.9/53.2	1.33	-0.20	4.9/34.5	2.78	-0.80	10.5/38.4	6.00	+1.10	8.4/27.3	4.80	+2.10	7.0/32.2	4.00	+2.70	7.0/30.8	4.00	+2.60	8.4/32.2	4.80	+4.20	7.0/29.4	1.43
	13	-0.70	13.3/46.9	7.60	-1.00	14.7/65.8	1.64	-0.20	4.9/34.5	2.78	-0.70	11.9/44.8	6.80	+1.10	9.8/26.6	5.60	+2.00	7.0/32.2	4.00	+2.70	7.0/31.5	4.00	+2.60	8.4/32.2	4.80	+4.40	8.4/32.2	1.71
Control	..	-0.60	4.2/21.0	2.14	-0.90	4.2/43.4	0.48	-0.20	4.2/21.0	2.14	-0.70	3.5/23.1	1.78	-0.70	3.5/23.1	1.78	-0.50	4.2/21.0	2.14	-0.60	4.2/22.4	2.14	-0.70	2.8/22.4	1.43	+1.40	4.2/22.4	0.91
B. welchii "L"	1	-0.60	9.1/34.3	4.65	-0.90	4.9/46.2	0.56	-0.20	4.9/32.1	2.50	-0.70	8.4/30.1	4.25	+1.00	7.7/28.0	3.93	+2.10	4.2/37.6	2.14	+2.50	6.3/34.3	3.21	+2.60	9.1/32.9	4.64	+3.60	5.6/21.0	1.21
	3	-0.80	12.6/46.2	6.43	-0.70	9.8/49.0	1.11	-0.20	4.9/32.1	2.50	-0.70	11.2/30.1	5.72	+1.00	7.7/28.0	3.93	+2.10	7.7/30.8	3.57	+2.90	7.0/33.6	3.57	+2.80	9.1/32.9	4.64	+3.60	8.4/25.2	1.82
	6	-0.80	13.4/56.0	7.86	-0.60	13.3/54.6	1.51	-0.20	5.6/35.2	2.86	-0.60	12.6/51.1	6.43	+1.10	8.4/30.1	4.28	+2.40	8.4/30.1	4.28	+2.90	7.0/33.6	3.57	+2.80	9.1/32.9	4.64	+3.60	11.9/28.5	2.58
	13	-0.70	13.3/53.9	6.78	-0.80	16.1/67.2	1.83	-0.20	5.6/35.2	2.86	-0.60	12.6/51.1	6.43	+1.00	9.8/32.9	5.00	+2.40	7.7/37.1	3.93	+2.90	7.0/33.6	3.57	+2.60	9.1/34.3	4.64	+4.50	12.6/39.2	2.73

Reaction: -- = alkaline to neutral red; + = acid to neutral red; cc normal acid or alkali per 100 cc of medium.

Ammonia and amino nitrogen expressed in milligrams per 100 cc of culture medium.

Control indicates initial composition of medium. Net results may be obtained by subtracting the control from the day's analysis.

Amino nitrogen is corrected in each instance for ammonia.

various groups, and it is confidently expected that the anaerobic bacilli may exhibit more or less characteristic metabolic curves which may be found eventually to possess value in their differentiation.

An important question arises: Will anaerobic bacteria under parallel laboratory conditions produce from year to year metabolic growth curves which are qualitatively and, within reasonable limits, quantitatively alike? It appears to be a fact that aerobic bacteria will react thus.<sup>22</sup> A study of the metabolism of culture 617 D, for the years 1917, 1918, and 1920 (see table 1) reveals the fact that this strain reacted in a very satisfactory manner. The curves are strikingly similar both with respect to daily transformations and cumulative nitrogenous changes in the mediums. It must be admitted that annual studies were not made of the remaining organisms identified as the Welch bacillus;<sup>23</sup> on the other hand, the general features were faithfully reproduced by each strain, and the general impression is that a single chemical type of organism is under consideration.<sup>24</sup>

*Origin of Cultures.*—Dr. Simonds isolated 617 D from an infected gunshot wound sustained by a Belgian soldier. It was used by Bull<sup>15</sup> for the production of the soluble toxin characteristic of the Welch bacillus. This culture may be regarded as the type organism of the Welch bacillus group.

Culture A was obtained from the National Research Council. The history is unknown except that the organism was isolated from a case of gas gangrene.

Culture B was isolated from an infected wound by Simonds.

Culture C was isolated from the feces of a dog.

Culture D was obtained from the National Research Council. Its origin is similar to that of culture A.

Culture E was obtained from laboratory dust.

Culture F was obtained from an infected tonsil.

Culture G was isolated from a case of gas gangrene, by Simonds.

Culture H was obtained from the feces of a horse.

Culture I was isolated from a gunshot wound.

Culture J was obtained from a case of "gas bacillus diarrhea."

Culture K was from a fatal case of emphysematous gas gangrene.

Culture L was obtained from Dr. Karl Meyer.

<sup>22</sup> Kendall, Day and Walker: *Ibid.*, 1913, 13, p. 425.

<sup>23</sup> Simonds' criteria (footnote 1) were followed as a standard.

<sup>24</sup> In subsequent publications, the chemical identity of the Welch bacillus will be contrasted with that of other anaerobic bacilli.

## DISCUSSION

All the cultures agree in the following cultural characteristics:<sup>25</sup> Gas and acid are produced in glucose, lactose, saccharose, glycerol and starch. Those cultures which were inoculated into maltose fermented it energetically. A stormy fermentation developed in milk. Gelatin was softened in every instance. The time required to bring about a chemical change of the gelatin protein of sufficient magnitude to prevent solidification when the medium was placed in the icebox for several hours varied from 2-13 days. As a general rule, the softening was distinct by the end of the third day of incubation, and it is of interest to find that the amino nitrogen content of the medium at the time of complete fluidification is increased only slightly above that of the uninoculated controls in each culture studied.

It would appear that the softening of the gelatin medium, referred to by Welch and Nuttall,<sup>2</sup> and noticed by many investigators, is plausibly associated with a change in the gelatin protein whereby it becomes so changed chemically that the physical property of solidification no longer takes place, but as the titratable acidity does not change materially, no definite conclusions can be drawn at this time of the significance of the phenomenon. It was not possible, however, to demonstrate the presence of a soluble gelatinase.<sup>26</sup> This is in sharp contrast to the parallel cultures of *Bacillus sporogenes*.

The ammonia formation (deamination) is greater in mediums containing protein and protein derivatives alone than in those containing both protein and utilizable carbohydrate. In this respect, the amino nitrogen and the ammonia nitrogen show a striking parallelism. As the ammonia appears to be a waste product, indicative of the intracellular utilization of protein,<sup>27</sup> by bacteria, it is not surprising to find that the "sparing action of utilizable carbohydrate for protein" is thus clearly indicated by the distinctly lesser formation of ammonia in those cultures containing utilizable carbohydrate.

The toxicity of the cultures studied was found to decrease rapidly after the first 24 hours of growth; this may possibly stand in relation to the gradual change in the protein molecule, but it is rather more probable that the soluble poison is associated with the period of great

<sup>25</sup> The characters referred to are those induced in mediums on which metabolism studies were made. Complete fermentation reactions will be recorded later.

<sup>26</sup> *Bacillus sporogenes* and other proteolytic anaerobes produce soluble proteolytic enzymes. These will be discussed in a later communication.

<sup>27</sup> Kendall and Walker: Jour. Infect. Dis., 1915, 17, p. 442.

numerical increase of the bacteria, and therefore "a growth product" rather than an "energy product," which would appear of course cumulatively as the culture grows older.

A noteworthy change in titratable acidity takes place in those mediums containing utilizable carbohydrates; the reaction increases markedly in acidity, even during the first 24 hours' growth. In many instances the maximum acidity is reached, or nearly reached, before the end of the third day, at which time the next series of determinations were made. This is in harmony with the almost explosive violence of the fermentation incited during the initial period of growth. The change of reaction in mediums containing no utilizable carbohydrate is little indeed, even in gelatin, in which a distinct but moderate action on protein (as shown by the formol titration) is indicated. Growth languishes in purely protein mediums although those rich in protein, and especially highly organized protein, are more favorable to development than those containing only protein derivatives, as meat extractives and peptones. *Bacillus welchii*, therefore, is not a proteophilic organism, and its characteristic activity is on utilizable carbohydrate.

Spores were always found in mediums containing no utilizable carbohydrate; they were never discovered in mediums in which active fermentation had taken place.

The organisms of the Welch group studied in this series produced the typical "stormy fermentation" in milk. The reaction is typically almost explosive in its violence. This phenomenon, due to a rapid decomposition of the lactose with the liberation of gas, and the formation of acid, takes place within the first 24 hours' incubation.<sup>28</sup> A large proportion of the milk sugar is transformed into nonfermentable products by this initial period of rapid development and reactivity. Subsequent development is slow and little additional change, aside from a slight increase in acidity, is demonstrable. The casein is rendered insoluble, owing in part at least to the accumulation of acid products of fermentation, and the insoluble mass is riddled with holes, presumably caused by the liberation of gas as the lactose enclosed in the coagulum is decomposed by the organism. The casein coagulum appears small in amount, suggesting at first sight that an actual

<sup>28</sup> If the milk is not freed from oxygen before inoculation, it not infrequently happens that the casein is coagulated while but little or no visible gas is formed. Simonds (footnote 1) was apparently the first observer to call attention to this peculiar behavior under such conditions.

proteolysis has taken place. A consideration of the nitrogenous changes induced in the medium, however, renders this explanation unlikely. The amino nitrogen decreases materially during the first day of incubation, and although the amount of amino acid may subsequently increase to a point above that of the uninoculated controls, it never reaches an amount indicative of a degree of proteolysis sufficient to account for more than minimal amounts of decomposition of casein. It might be assumed, of course, that the gas bacilli utilized the products of degradation of the protein constituents of the milk, thus masking the chemical evidence of digestion; the very small amount of ammonia formed during the process is distinctly against this possibility, and, in addition, the mediums containing no utilizable carbohydrate (plain broth and gelatin) do not exhibit reactions indicative of more than minimal attack upon proteins. It would appear, therefore, that the gas bacillus is an organism characterized by minimal proteolytic powers, but possessed of unusually vigorous fermentative powers.

Available evidence indicates that alcohols of the hexose series, sorbitol, mannitol, and dulcitol, are not attacked by *B. welchii*.<sup>1, 5</sup> The hexose sugars, glucose, fructose, mannose and galactose, on the contrary, appear to be energetically attacked. This would suggest that the aldose configuration—CHO,—but not the alcohol configuration—CH<sub>2</sub>OH—affords a point of attachment, permitting the endo enzymes of the organism to decompose the aldose molecules, but not the corresponding alcohols. Sucrose, which possesses no free aldehyde grouping, is readily fermented, however, but the possibility or even probability of a preliminary hydrolysis of the sucrose molecule to glucose and fructose must be borne in mind in this instance. Many strains ferment glycerol, a triatomic alcohol, without difficulty; hence, judgment must be withheld concerning the relations between aldehyde and alcohol groupings in determining utilizability of the hexoses until much more detailed studies on complete series of hexose sugars and their derivatives can be made. For the present, however, the non-fermentability of the alcohols derived from the more commonly available hexoses is a point of distinction in the cultural reactions of the gas bacillus.

#### SUMMARY

*B. welchii* represents a type of widely distributed and closely related anaerobic bacilli which exhibit in common the ability to induce a vigorous fermentation of the commonly used carbohydrates. The

alcohols of the hexose sugars are, so far as known, not utilizable as sources of energy by the members of the gas bacillus group. Glycerol is attacked by many strains. Growth is relatively feeble in nonsaccharine mediums. Gelatin, which is a better substrate than plain broth for the development of the organism in the absence of utilizable carbohydrates, is so altered by the microbes that it will no longer solidify. This softening is not due to the action of a soluble proteolytic enzyme, as is the case with *B. sporogenes* and other strongly proteolytic anaerobes. The nitrogenous changes in the medium measurable by available methods are of insufficient magnitude to afford a clear-cut, satisfactory explanation of the phenomenon on the basis of nitrogenous decomposition. Little free ammonia is formed, indicating little endogenous utilization of protein. There is a distinct, although moderate, increase of amino nitrogen in the gelatin medium incidental to growth, however, and it is by no means impossible that this amino nitrogen increase, representing the resultant of protein cleavage by the organisms and the unused residue of this cleavage, may be so related to the gelatin molecule that the latter no longer possesses the chemical and physical properties necessary to exhibit the characteristic ability to solidify on cooling. The change in titratable acidity, it should be remarked, is small indeed in purely protein mediums.

The sparing action of utilizable carbohydrates for protein is clearly indicated in the analytic tables.

The most characteristic reaction of the gas bacillus group is in milk. The "stormy fermentation," the slightly pink color of the casein coagulum, the riddled appearance of the latter, and the distinct odor of butyric acid are the significant features. No other group of anaerobic bacteria, so far described, exhibits this cultural complex in its entirety. It may be stated that mixed cultures of bacteria inoculated into milk which has been freed from oxygen and heated to 80 C. for 20 minutes prior to incubation, which exhibit the characteristic stormy fermentation within 18 hours, contain members of the *B. welchii* group. Material from infected tissues does not, except under unusual conditions, contain gas bacillus spores. Hence, the heating to 80 C. must be dispensed with to obtain results with the milk test. In doubtful cases, the Welch-Nuttall rabbit test<sup>29</sup> will almost always yield a positive result. Subcultures from the liver<sup>29</sup> of such animal will furnish active subcultures although not necessarily in a state of purity.

<sup>29</sup> Kendall and Smith: Boston Med. & Surg. Jour., 1910, 158, p. 578; Arch. Pediatrics, 1911, 28, p. 389.

## VIBRION SEPTIQUE

## STUDY XLV

The first organism belonging to the group of anaerobic bacteria was isolated by Pasteur<sup>1</sup> and described by him under the designation *Vibrion septique*. A few years later Koch<sup>2</sup> isolated an anaerobic bacillus from garden soil which, on injection into experimental animals, gave rise to marked edema at and near the site of inoculation. Koch pointed out some relatively minor pathologic differences exhibited by his organism in contrast to *Vibrion septique*, and named it the bacillus of malignant edema; in bacteriologic terminology, *Bacillus oedematis maligni*. The latter name has supplanted largely the original term *Vibrion septique*, but the reasons for so doing do not appear to be convincing. From the standpoint of terminology, neither name is correct. Also, these original studies were made under conditions which make it almost certain that pure cultures were unattainable. In spite of these cultural difficulties, however, the characteristics of the organism, and more particularly the nature of the lesions induced in laboratory animals, are of sufficient definiteness to furnish a satisfactory means of comparison of existing strains with those of Pasteur and Koch. There appears to be unequivocal evidence that *Vibrion septique* and *B. oedematis maligni* are to be regarded as identical, and also that the same organism is identifiable among current strains.

The name *Vibrion septique* is preferable, therefore, to *B. oedematis maligni*, pending the time when bacteriologic etymologists shall confer a final and correct designation on the microbe. Its use recalls the genius of Pasteur who added to the phenomena of living things the conception of anaerobic existence.

Descriptions of *Vibrion septique* differ markedly. The earlier observers ascribed proteolytic powers of considerable magnitude to the organism, and even at the present time there is confusion on this point. The most carefully controlled work on this organism, however, by Miss Robertson, Meyer, and others has failed to demonstrate any evidence of significant action on protein.<sup>3</sup> On the contrary, carbohydrates are energetically decomposed.

<sup>1</sup> Pasteur and Joubert: *Bull. de l'Acad. méd.*, 1877, 6, p. 781.

<sup>2</sup> Mitt. a. d. kais. Gesundheitsamte, 1881, 1, p. 48.

<sup>3</sup> See Weinberg and Séguin: *La Gangrène Gazeuse*, Paris, 1917. Medical Research Committee, Report No. 39, London, 1919, contains literature on the subject.



*Vibrio septique* became conspicuous during the last half decade as the second most important incitant of fulminating gas gangrene. It is chiefly due to the activities of the organism as a dangerous contaminant of wounds of warfare, and of contused wounds in general, that so much study has been expended on it in recent years. The morphologic, cultural and serologic peculiarities have been thoroughly examined<sup>4</sup> and the nature of the poison has received much attention.<sup>3, 5</sup> The biochemistry, however, in connection with that of other anaerobic bacteria, has not been the subject of study, with the exception of the observations of Wolf.<sup>6</sup> Wolf's studies, in essence, emphasize the following peculiarities of the chemistry of *Vibrio septique*.

1. The organism produces a brilliant red coloration in the cooked meat medium. This is to be contrasted with a paler pink color produced by several anaerobes grown in protein-carbohydrate mediums, as for example, milk.<sup>7</sup>

2. The fermentation reactions of *Vibrio septique* show points of resemblance to those of *B. welchii*, especially with reference to intensity. The associated nitrogenous changes, however, are quantitatively different, *Vibrio septique* producing somewhat less gas than *B. welchii*, and also the amount of ammonia accumulating in cultures being less. The amino nitrogen, on the contrary, is relatively greater under approximately parallel conditions.

3. *Vibrio septique* produces much gas in milk. The total volume may exceed the amount of fermented medium 2.5 times. The rate of evolution of the gas, however, is much slower than that of the *Welch bacillus*.

4. *Vibrio septique* is carbohydophilic rather than proteophilic; in this respect it resembles the *Welch bacillus* very closely. Wolf's experiments disclose the sparing action of utilizable carbohydrate for protein in cultures of the organism containing sugars.

The soluble poison of *Vibrio septique*, like that of *B. welchii*, appears to be associated with the growth of the organism rather than with the period when the products of metabolism are at their maximum. Indeed, after the first day of growth the potency of the poison, like that of the *Welch bacillus*, decreases rapidly. The nature of the soluble

<sup>4</sup> Miss Robertson: *British Med. Jour.*, 1918, I, p. 583. Henry: *Jour. Path. & Bacteriol.*, 1916-1917, 21, p. 344. Meyer: *Jour. Infect. Dis.*, 1915, 17, p. 458.

<sup>5</sup> Miss Robertson: *Jour. Path. & Bacteriol.*, 1920, 23, p. 153.

<sup>6</sup> *Ibid.*, 1918, 22, p. 115.

<sup>7</sup> Simonds: *Monograph 5*, Rockefeller Institute, 1915, p. 40.

poison is wholly unknown, but it appears that specific serums, containing substances capable of neutralizing it, and therefore possessed of some curative value, have been prepared.<sup>3, 5</sup> As this poison, however, is maximal during the early hours of growth of the organism, there is nothing in the ordinary metabolic chemistry of *Vibrio septique* which would be of significance in elucidating its nature or potency. Admixture with other organisms appears to prevent the formation of specific antibodies for *Vibrio septique*,<sup>5</sup> and prolonged incubation definitely reduces the content of filtrates of cultures in poisonous substances. Filtrates of young cultures, on the contrary, are relatively stable with reference to their poisonous properties. These facts suggest not only that the poisonous principle is formed during the period of rapid multiplication of the organisms, and therefore a labile substance formed as a waste product incidental to the transformation of protein (or protein derivatives) for structural purposes rather than a product of the metabolism of protein for energy. It also suggests that the further growth of *Vibrio septique*, or of contaminating bacteria, destroys the potency of the poisonous substance.

The cultural identification of *Vib. septique* presents some difficulty. The close resemblance of the organism to the Welch bacillus has undoubtedly led to confusion in the past, and even at the present time authorities are not in complete accord in the points of difference between these microbes.

It is claimed by many<sup>8</sup> that *Vibrio septique* fails to ferment saccharose, differing in this respect from the Welch bacillus and *Bacillus fallax*. On the other hand, there are several observers who have apparently found that saccharose is fermented. Of the eight cultures studied in the series presented below, a majority fermented saccharose with the formation of gas and acid, although the amount of gas evolved and the rate of evolution were distinctly slower than was the case with the more readily attacked carbohydrates, as glucose, lactose and maltose. Glycerol and starch were not fermented by any of the strains studied, and the evolution of gas in milk was much slower than that characteristic of cultures of the Welch bacillus. These appear to be points of difference between the two organisms which may possess more than academic value in their cultural recognition. It is by no means impossible, or even improbable, that the saccharose-ferment-

<sup>8</sup> Miss Robertson: *British Med. Jour.*, 1918, I, p. 583. Achalme: *Ann. Inst. Past.*, 1902, 16, p. 633. Distaso and Jungano, *Les Anaérobies*, 1910, p. 78. Medical Research Committee, Report 39, 1919, p. 23.





ing varieties may be of the same general significance as the corresponding types of the colon bacillus. If such prove to be the case, the bacteriologist of the future will recognize distinct types of *Vibrio septique*, precisely as the types of the gas bacillus are now recognized.<sup>4, 7</sup>

The cultures studied in this series comprised the following:

*Vibrio septique*, culture A—from the National Research Council. Obtained originally from an infected wound.

*Vibrio septique*, culture B—from the same source.

*Vibrio septique*, culture C—from Dr. Karl Meyer.

*Vibrio septique*, culture D—from Dr. Karl Meyer.

*Vibrio septique*, culture E—from the Army Medical School.

*Vibrio septique*, culture F—from the Army Medical School.

*Vibrio septique*, culture G—a stock laboratory culture.

*Vibrio septique*, culture H—from a culture originally in the Pasteur Institute.

The organisms were purified by the method of single cell isolation described previously.<sup>9</sup> The general plan of inoculation and chemical examination will be found in the study of the Welch bacillus.<sup>10</sup>

#### DISCUSSION

The analytic figures are self-explanatory. The quantitative changes in the nitrogenous constituents of the different mediums are very similar for the eight organisms studied. This suggests that the various strains may be properly regarded as of one general type. Culture H alone failed to ferment saccharose with the production of gas and acid. The possible significance of this characteristic has been commented on in the foregoing. The inability of all of the strains to induce visible signs of fermentation in glycerol and starch, and the relatively slow accumulation of acid and gas in milk cultures, points to a distinct departure, chemically considered, from the corresponding changes induced by the Welch bacillus.<sup>10</sup> Final judgment should be withheld on the validity of these features as points of distinction between *Vibrio septique* and the Welch bacillus, until a considerable number of independent studies by various observers have been made. The difficulties attending the purification and study of anaerobic bacteria with existing methods make sweeping statements regarding them of precarious value.

<sup>9</sup> Kendall, Ryan and Cook: *Jour. Infect. Dis.*, 1921, 29, p. 227.

<sup>10</sup> Kendall, Day and Walker: Study XLIV, *Jour. Infect. Dis.*, 1922, 30, p. 141.

The ammonia formation indicative of the intracellular utilization of protein or of protein derivatives for energy,<sup>11</sup> is little indeed, even in cultures, such as gelatin, from which the combined energy and structural requirements must be derived from nitrogenous sources. In this respect, the organism resembles the Welch bacillus.<sup>10</sup> The amino nitrogen accumulation also is minimal, both in protein and carbohydrate-protein mediums. This is quantitatively in contrast with the Welch bacillus, where it was found, in the thirteen strains studied, that the amino nitrogen accumulation was somewhat greater than the ammonia nitrogen formation. This observation is in contrast to that of Wolf,<sup>8</sup> who found that his strain identified as the *Vibrio septique* produced a gradually increasing amount of amino nitrogen, whereas his strain of the Welch bacillus (*B. perfringens*) produced minimal amounts of amino nitrogen.

As Wolf stresses this difference as distinctive, it would appear either that there is disagreement on the identification of the respective organisms, or that the differences are of such small magnitude as to be subject to environmental influences that would lead to slight variations in the amounts of amino nitrogen consumed by the organism, thus influencing the residual amino nitrogen which is measured in the cultural mediums. In this respect, so far as the published descriptions of Wolf's organisms show, the fundamental distinctions between his *B. perfringens* and *Vibrio septique* appear to agree with the criteria presented for *B. welchii* and *Vibrio septique* in this series. As the determination of amino nitrogen represents merely free  $\text{NH}_2$  groups in protein, peptone or polypeptids, and not the total nonprotein nitrogen, this difference may be of academic value only. A much more valuable determination of nitrogenous changes in cultural mediums, now determined as amino nitrogen, would be a differentiation into protein and polypeptid nitrogen. Unfortunately this is not possible at the present time.

Gelatin was softened by each strain of *Vibrio septique* studied. The softening, however, was not due to a detectable soluble enzyme, and the slight change in the nitrogenous constituents of the gelatin suggests strongly that the organism induces only slight decomposition of the medium by the organism. *Vibrio septique* is not an organism with marked proteolytic powers. In this respect it is in perfect accord with the Welch bacillus.

<sup>11</sup> Kendall and Walker: *Ibid.*, 1915, 17, p. 442.

The organisms studied evolved considerable amounts of gas from the lactose of milk, but the generation of gas was slow and entirely different in character from the almost explosive action of the gas bacillus under similar conditions. The casein was not riddled with holes, as is the case with typical Welch bacillus cultures, and the coagulum appears to be more voluminous. The bright red coloration which gradually develops is more striking than the faint, reddish brown color of typical gas bacillus casein coagula. There is no evidence of more than minimal action on the proteins of the milk, as evidenced by ammonia production and amino acid accumulation.

The reaction of purely protein mediums undergoes little change; in mediums containing utilizable carbohydrate, however, the reaction becomes quite strongly acid.

#### SUMMARY

*Vibrio septique* is a carbohydrophilic anaerobic bacillus, which decomposes utilizable carbohydrates energetically with the formation of considerable amounts of titratable acid and the evolution of considerable gas.

It softens gelatin, but without visible evidences of energetic action on the protein of the medium. Gas and acid are generated in milk cultures, and the casein coagulum becomes vividly pink. The nitrogenous changes in milk are minimal, suggesting that the principal change (energy change) is at the expense of the lactose. The nature and extent of the visible changes in milk, however, are quantitatively distinctly less than those characteristic of the Welch bacillus.

*Vibrio septique* possesses many points of resemblance to the Welch bacillus. The cultures studied differ from the Welch bacillus in their nonability to ferment visibly glycerol or starch; also, the action on saccharose is distinctly less than that on the other carbohydrates studied, or is absent.

These characteristics, taken in connection with the relatively slow fermentation of lactose in milk cultures, appear to be distinct points of difference between the two organisms.

It would appear that the saccharose fermenting and nonsaccharose fermenting strains comprise two distinct types, parallel in significance to the four types of Welch bacillus defined by Simonds<sup>7</sup> and so accepted by Henry.<sup>4</sup>



## BACILLUS FALLAX

## STUDY XLVI

*Bacillus fallax* is a rather small anaerobic bacillus, occurring singly or in pairs, which was first isolated from infected wounds and described by Weinberg and Séguin.<sup>1</sup> The organism has occasionally been recovered from the blood stream of the patient during the earlier days of the infection. The organism is distinctly more slender than most of the gunshot wound microbes, and the larger axis is frequently distinctly curved, when observed in stained preparations derived from actively growing cultures. These observations have received confirmation in the studies of Henry<sup>2</sup> and others.<sup>3</sup>

A majority of investigators have directed attention to the comparative infrequency with which spores are observed in culture mediums. This is in distinct contrast to the readiness with which other anaerobic organisms sporulate under parallel conditions. Generally speaking, anaerobic bacteria, or indeed any bacteria, rarely produce spores in the tissues of animals or of man; also, anaerobes with the possible exception of the vigorous proteolytic microbes, such as *B. sporogenes*, fail to form spores in mediums containing utilizable carbohydrates.

*B. fallax* appears to be somewhat more exacting in regard to the environmental conditions governing sporulation than other organisms of the anaerobic group thus far studied, but it may be said that the cultures studied in the series reported in the following, and identified as *B. fallax*, formed spores consistently but not abundantly in mediums containing protein, such as gelatin or blood serum, in which the reaction remains at or near the neutral point. Spores were never observed in mediums containing utilizable carbohydrates, irrespective of the protein constituents of the medium.

The pathologic and cultural characteristics of *B. fallax* have been studied by Weinberg and Séguin,<sup>4</sup> and by Henry.<sup>2</sup> The most significant feature of the cultural complex is the apparent inability of the organism to utilize lactose. The organisms of the series reported herein are not in accord in this respect with those of Henry. A slow

<sup>1</sup> Compt. rend. Soc. biol., 1915, 78, p. 686; 1916, 79, p. 581.

<sup>2</sup> Jour. Path. & Bacteriol., 1916, 21, p. 344.

<sup>3</sup> Medical Research Committee, Report 39, 1919.

<sup>4</sup> La Gangrène Gazeuse, 1917.

evolution of gas in milk (utilization of lactose) with no concurrent evidence of proteolysis is in harmony with this view.

The production of gas in starch mediums is also an important cultural and diagnostic feature. The cultural complex suggests strongly that *B. fallax* is culturally similar to the gas bacillus (Welch bacillus) in that the hexoses and the better known bioses (lactose and saccharose) are fermented, while the alcohols of the hexose series, especially mannitol, are unattacked. The intensity of fermentation, both with respect to rate and amount, is decidedly less vigorous than that of the Welch bacillus, however. The organism is carbohydrophilic. Its action on protein is minimal.

Four cultures are studied in this series. They were obtained from the following sources:

Culture A—National Research Council, from an infected war wound.

Culture B—From the National Research Council, labeled "Gas Bacillus, from an infected wound."

Culture C—From the intestinal contents of a man suffering from an acute diarrhea.

Culture D—From intestinal contents.

The method of purification by the modified Barber single cell method and the general procedure followed in the study of the metabolism of these cultures is that described previously.<sup>5</sup>

#### RESULTS

*Nitrogenous Changes.*—The extremely small amounts of ammonia formed during the growth of *B. fallax* in cultural mediums show conclusively that the action of the organism on protein is minimal. It is not proteophilic. Even after 2 weeks' growth the ammonia has not increased to the extent of 15 mg. in 100 cc of culture medium in gelatin or in plain nutrient bouillon in which no utilizable carbohydrate is present, and in which, consequently, the greatest evidence of proteolysis might confidently be looked for. The change in amino-nitrogen, similarly, is equally insignificant.

The addition of glucose, saccharose, or starch results in a decided increase of activity, which is manifested by a moderate evolution of gas, principally CO<sub>2</sub> and H<sub>2</sub>, and the gradual accumulation of titratable acid. The increase of acidity is greatest during the first 24 hours of growth. Lactose is less rapidly decomposed, and the total amount of gas is distinctly less than that of the other carbohydrates mentioned.

<sup>5</sup> Kendall, Ryan and Cook: Jour. Infect. Dis., 1921, 29, p. 227. Kendall, Day and Walker: Studies in Bacterial Metabolism, XLIV, XLV, *ibid.*, 1922, 30, pp. 141 and 155.

TABLE 1  
BACILLUS FALAX

Day		Plain			Gelatin			Glucose			Mannitol			Glycerol			Lactose			Saccharose			Starch			Milk				
		Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen		
Control.....	Control A....	-0.50	3.5/23.1	2.08	-0.50	5.6/35.7	0.62	-0.30	3.5/21.7	2.08	-0.50	3.5/23.1	2.08	-0.50	3.5/23.1	2.08	-0.50	3.5/23.1	2.08	-0.40	3.5/23.1	2.08	-0.40	3.5/21.7	2.08	-0.40	3.5/21.7	2.08	-0.40	3.5/21.7
	1	-0.50	4.2/23.8	2.50	-0.40	5.6/36.4	0.62	+3.00	3.5/21.7	2.08	-0.40	4.2/22.4	2.50	+2.20	4.2/23.1	2.50	+0.90	4.2/22.4	2.50	+2.50	4.2/21.0	2.50	+2.50	4.2/21.0	2.50	+2.50	4.2/21.0	2.50	+2.50	4.2/21.0
	3	-0.50	4.2/23.8	2.50	-0.30	5.6/37.8	0.62	+3.10	3.5/21.7	2.08	-0.40	4.2/22.4	2.50	+2.30	4.2/23.1	2.50	+2.50	4.2/22.4	2.50	+2.40	4.2/22.4	2.50	+2.50	4.2/21.0	2.50	+2.50	4.2/21.0	2.50	+2.50	4.2/21.0
	6	-0.55	4.2/26.6	2.50	-0.20	5.6/38.4	0.62	+3.10	4.2/21.7	2.50	-0.40	4.2/22.4	2.50	+2.20	4.2/23.1	2.50	+2.50	4.2/22.4	2.50	+2.40	4.2/22.4	2.50	+2.50	4.2/21.0	2.50	+2.50	4.2/21.0	2.50	+2.50	4.2/21.0
	13	-0.55	4.2/26.6	2.50	-0.30	5.6/40.6	0.62	+2.80	4.2/22.4	2.50	-0.30	4.2/22.4	2.50	+2.20	4.2/23.1	2.50	+2.50	4.2/23.1	2.50	+2.40	4.2/23.1	2.50	+2.50	4.2/21.0	2.50	+2.50	4.2/21.0	2.50	+2.50	4.2/21.0
	21	-0.60	4.2/26.6	2.50	-0.30	5.6/40.6	0.62	+2.80	4.2/22.4	2.50	-0.40	4.2/22.4	2.50	+2.20	4.9/23.1	2.92	+2.50	4.2/23.1	2.50	+2.40	4.9/23.1	2.92	+2.80	4.2/21.0	2.50	+3.40	5.6/23.8	1.17	+3.40	5.6/23.8
Control.....	Control B....	-0.45	3.5/23.1	1.85	-0.80	4.9/44.1	0.54	-0.20	3.5/21.7	1.85	-0.40	3.5/23.1	1.85	-0.40	3.5/23.1	1.85	-0.40	3.5/23.1	1.85	-0.50	3.5/23.1	1.85	-0.40	3.5/23.1	1.85	-0.40	3.5/23.1	1.85	-0.40	3.5/23.1
	1	-0.50	3.5/22.4	1.85	-0.80	7.0/46.2	0.77	+2.40	4.2/21.7	2.22	-0.30	4.2/25.2	2.22	-0.60	3.5/22.4	1.85	+2.60	3.5/24.5	1.85	+2.90	4.2/22.4	2.22	+2.80	3.5/23.1	1.85	+2.80	3.5/23.1	1.85	+2.80	3.5/23.1
	3	-0.50	4.2/23.8	2.22	-0.70	7.7/46.9	0.85	+2.80	4.2/21.7	2.22	-0.40	4.2/25.9	2.22	-0.70	3.5/23.1	1.85	+2.80	4.2/23.8	2.22	+3.10	4.2/23.1	2.22	+3.00	4.2/23.8	2.22	+3.00	4.2/23.8	2.22	+3.00	4.2/23.8
	6	-0.50	4.2/23.8	2.22	-0.50	7.7/46.9	0.85	+2.70	4.2/22.4	2.22	-0.40	4.9/24.5	2.59	+0.70	3.5/23.1	1.85	+2.90	4.2/24.5	2.22	+3.10	3.5/23.8	2.22	+2.90	4.2/23.8	2.22	+2.60	8.4/24.5	1.60	+2.60	8.4/24.5
	13	-0.50	4.9/25.9	2.50	-0.40	7.7/47.6	0.85	+2.60	4.9/22.4	2.59	-0.40	4.9/25.2	2.59	+0.80	4.2/23.8	2.22	+2.40	4.2/24.5	2.22	+2.70	4.9/24.5	2.59	+3.00	4.9/23.8	2.59	+3.00	4.9/23.8	2.59	+3.00	4.9/23.8
	21	-0.50	5.6/27.3	2.96	-0.40	8.4/47.6	0.93	+2.70	4.9/23.1	2.59	-0.40	4.9/25.2	2.59	+0.80	4.2/23.8	2.22	+2.40	4.2/24.5	2.22	+2.70	4.9/24.5	2.59	+3.00	5.6/24.5	2.59	+3.00	5.6/24.5	2.59	+3.00	5.6/24.5
Control.....	Control C....	-0.40	2.1/21.7	1.25	-0.40	3.5/41.3	0.39	-0.20	2.8/19.6	1.66	-0.40	2.1/23.1	1.25	-0.40	2.8/21.7	1.66	-0.40	2.8/20.3	1.66	-0.40	2.1/20.3	1.25	-0.40	3.5/20.3	2.08	-0.40	3.5/20.3	2.08	-0.40	3.5/20.3
	1	-0.50	2.8/22.4	1.66	-0.60	3.5/41.3	0.39	+2.60	2.1/21.0	1.25	-0.50	4.2/22.4	2.50	+2.10	2.1/20.3	1.25	+2.60	2.8/20.3	1.66	+3.00	2.1/20.3	1.25	+2.80	2.8/21.0	1.66	+3.00	2.8/21.0	1.66	+3.00	2.8/21.0
	3	-0.50	2.8/22.4	1.66	-0.60	4.2/41.3	0.46	+2.80	2.1/21.0	1.25	-0.45	3.5/22.4	2.08	+2.00	2.1/20.3	1.25	+2.60	2.1/22.4	1.25	+3.10	2.8/20.3	1.66	+2.90	2.8/21.0	1.66	+3.00	2.8/21.0	1.66	+3.00	2.8/21.0
	6	-0.50	2.8/22.4	1.66	-0.60	4.2/41.3	0.46	+3.00	2.8/21.0	1.66	-0.45	3.5/23.8	2.08	+2.10	2.8/20.3	1.66	+2.70	2.8/22.4	1.66	+3.20	2.1/21.0	1.25	+2.80	2.8/21.0	1.66	+3.10	2.8/21.0	1.66	+3.10	2.8/21.0
	12	-0.55	4.2/23.8	2.50	-0.50	4.9/42.0	0.54	+3.10	3.5/19.6	2.08	-0.60	3.5/23.8	2.08	+2.10	2.8/20.3	1.66	+2.70	3.5/21.7	2.08	+3.10	2.8/21.0	1.66	+3.10	3.5/21.0	2.08	+3.20	2.8/21.0	2.08	+3.20	2.8/21.0
	21	-0.55	4.2/23.8	2.50	-0.50	4.9/42.0	0.54	+3.10	3.5/19.6	2.08	-0.60	3.5/23.8	2.08	+2.10	2.8/20.3	1.66	+2.70	3.5/21.7	2.08	+3.10	2.8/21.0	1.66	+3.10	3.5/21.0	2.08	+3.20	2.8/21.0	2.08	+3.20	2.8/21.0
Control.....	Control D....	-0.40	2.1/21.7	1.25	-0.40	3.5/41.3	0.39	-0.10	2.8/19.6	1.66	-0.40	2.1/23.1	1.25	-0.40	2.8/21.7	1.66	-0.40	2.1/20.3	1.25	-0.40	2.1/20.3	1.25	-0.40	3.5/20.3	2.08	-0.40	3.5/20.3	2.08	-0.40	3.5/20.3
	1	-0.40	2.8/23.1	1.66	-0.40	2.8/41.3	0.31	+1.10	2.8/19.6	1.66	-0.50	2.8/21.7	1.66	-0.40	3.5/21.7	2.08	+0.80	2.1/21.0	1.25	+1.00	2.8/21.0	1.66	+1.40	2.8/21.7	1.66	+1.40	2.8/21.7	1.66	+1.40	2.8/21.7
	3	-0.50	3.5/22.4	2.08	-0.50	4.9/39.2	0.54	+1.10	2.8/19.6	1.66	-0.40	2.8/23.1	1.66	-0.40	3.5/21.7	2.08	+0.80	2.1/21.0	1.25	+1.30	2.8/21.0	1.66	+1.30	3.5/21.7	1.66	+1.40	2.8/21.7	1.66	+1.40	2.8/21.7
	6	-0.50	4.9/21.0	2.91	-0.50	5.6/40.6	0.62	+1.30	3.5/21.0	2.08	-0.35	3.5/23.8	2.08	-0.30	3.5/20.3	1.66	+1.60	2.1/21.7	1.25	+1.50	2.8/21.0	1.66	+1.60	2.8/21.7	1.66	+1.40	4.2/22.4	2.50	+1.40	4.2/22.4
	12	-0.55	5.6/21.0	3.33	-0.50	4.9/40.6	0.54	+1.30	3.5/20.3	2.08	-0.35	3.5/23.8	2.08	-0.20	4.9/21.7	2.91	+1.50	2.8/22.4	1.66	+1.30	3.5/21.7	2.08	+1.30	3.5/21.7	2.08	+1.30	3.5/21.7	2.08	+1.30	3.5/21.7
	21	-0.55	5.6/21.0	3.33	-0.50	4.9/40.6	0.54	+1.30	3.5/20.3	2.08	-0.35	3.5/23.8	2.08	-0.20	4.9/21.7	2.91	+1.50	2.8/22.4	1.66	+1.30	3.5/21.7	2.08	+1.30	3.5/21.7	2.08	+1.30	3.5/21.7	2.08	+1.30	3.5/21.7

Reaction: - = alkaline to neutral red; + = acid to neutral red; c = normal acid or alkali per 100 c.c. of medium.

Ammonia and amino nitrogen expressed in milligrams per 100 c.c. of culture medium.

Control indicates initial composition of medium. Net results may be obtained by subtracting the control from the day's analysis. Amino nitrogen is corrected in each instance for ammonia.

In milk, as well as lactose broth, however, there is a slow evolution of gas, and this factor, in association with the distinct and rapid development of an acid reaction, would appear to afford conclusive evidence that *B. fallax* is to be regarded as a lactose fermenter.

Cultures A and B fermented glycerol with moderate intensity. Cultures B and D failed to produce gas, but the reaction became somewhat acid, greater than the acidity developed in corresponding mediums containing no utilizable, non-nitrogenous source of carbon. Available evidence fails to furnish an adequate explanation for this phenomenon. It cannot be stated at this time whether Culture B is a nonglycerol fermenting variant of *B. fallax*, or whether a slight and unrecognized impurity in the glycerol furnished a small amount of substance utilizable for energy in place of the nitrogenous constituents, giving rise to this slight increase in acid above that characteristic of nonsaccharine mediums. The indifferent nitrogenous changes which characterize the proteolytic activities of the bacillus are insufficient to distinguish between these possibilities. Attempts to demonstrate a soluble, proteo-clastic enzyme were wholly unsuccessful. As the gelatin medium failed to soften even after two weeks' incubation, however, this negative result is to be expected.

#### SUMMARY

*B. fallax* is a carbohydrophilic organism, whose general cultural properties are reminiscent in a moderate degree of those characteristic of *B. welchii*.

The fermentation of starch with the production of gas and acid appears to be a mark of resemblance to the Welch bacillus, and a point of differentiation from the other anaerobic bacteria which comprise the flora of infected wounds of warfare.

The relatively gradual evolution of gas, both in milk and in mediums containing utilizable carbohydrates, contrasts markedly with the rapid generation of gas in cultures of the Welch bacillus.

Lactose is decomposed more slowly than the other carbohydrates which are utilizable as sources of energy by *Bacillus fallax*. This appears to be a feature of considerable diagnostic importance.

The coagulum formed gradually in milk as a result of the slowly increasing acidity attributable to the fermentation of the lactose is quite unlike that characteristic of *B. welchii* and *Vibrio septique*. It lacks the ragged, torn appearance characteristic of the former, and it fails to exhibit the reddish coloration of the latter.

## BACILLUS TERTIUS

## STUDY XLVII

*Bacillus tertius* was isolated from infected war wounds and described by Henry.<sup>1</sup> It received its name because it was encountered third in point of frequency of occurrence among the anaerobic bacteria of wounds by Henry. The morphology, especially of young, rapidly growing cultures, is fairly distinctive. Spores are formed readily in protein mediums, which are always of the plectridial type, and oval in outline when fully mature. Immature spores, which may be stained by Gram's method, appear first as small, deeply staining enlargements on one end of the bacillary rods. As the spore matures, it becomes longer, larger, and tends to lose its ability to stain with ordinary dyes. Eventually the mature spore is nearly twice the diameter of the parent rod, distinctly longer, and stainable only by intensified methods of coloration, as for example, steaming with carbol fuchsin. Once stained, however, the mature spore retains the color tenaciously; in this respect, it resembles spores of other organisms. The peculiarity is the frequency with which immature polar spores may be detected in actively growing cultures in the earlier stages of incubation.

The organism has been identified by Miss Robertson<sup>2</sup> as indistinguishable from *Bacillus* III of Rodella<sup>3</sup> and as *Bacillus* IX of von Hibler.<sup>4</sup> It may be closely related to the organism described by Fleming as *Bacillus* Y,<sup>5</sup> but this is doubtful because the latter is said to decompose protein slowly. Published descriptions by Henry<sup>1</sup> and others are in accord in ascribing carbohydrophilic, but not proteolytic properties to *B. tertius*.

An important cultural diagnostic feature is the fermentation of mannitol by the organism. In general, the alcohol derivatives of the hexoses do not appear to be utilizable as sources of energy by the saccharolytic anaerobic organisms; mannitol, however, is fermented by *B. tertius*, but dulcitol is unattacked. The organisms identified as *B. tertius* in the series presented in the following agree in this detail with the organism originally described.

<sup>1</sup> Brit. Med. Jour., 1917, 1, p. 806; Jour. Path. & Bacteriol., 1916, 21, p. 344.

<sup>2</sup> Ibid., 1915, 20, p. 327.

<sup>3</sup> Ztschr. f. Hyg., 1902, 39, p. 201.

<sup>4</sup> Untersuchungen über die pathogenen Anaëroben, u. s. w., 1908.

<sup>5</sup> Lancet, 1915, 2, p. 376.

TABLE 1  
BACILLUS TERTIUS

Day	Plain			Gelatin			Glucose			Mannitol			Glycerol			Lactose			Saccharose			Starch			Milk		
	Reaction	Amino Nitrogen	Percentage Amino Nitrogen to Total Nitrogen	Reaction	Amino Nitrogen	Percentage Amino Nitrogen to Total Nitrogen	Reaction	Amino Nitrogen	Percentage Amino Nitrogen to Total Nitrogen	Reaction	Amino Nitrogen	Percentage Amino Nitrogen to Total Nitrogen	Reaction	Amino Nitrogen	Percentage Amino Nitrogen to Total Nitrogen	Reaction	Amino Nitrogen	Percentage Amino Nitrogen to Total Nitrogen	Reaction	Amino Nitrogen	Percentage Amino Nitrogen to Total Nitrogen	Reaction	Amino Nitrogen	Percentage Amino Nitrogen to Total Nitrogen	Reaction	Amino Nitrogen	Percentage Amino Nitrogen to Total Nitrogen
Control.....	-0.40	2.1 21.7	1.25	-0.40	3.5 41.3	0.38	-0.10	2.8 19.6	1.68	-0.40	2.1 23.1	1.25	-0.40	2.8 21.7	1.68	-0.40	2.1 20.3	1.25	-0.40	2.1 20.3	1.25	-0.40	3.5 20.3	2.08	-0.40	2.8 25.2	0.59
Culture A....	-0.50	2.8 22.4	1.68	-0.60	4.8 42.7	0.34	+0.30	2.8 19.6	1.68	+1.50	2.8 21.7	1.68	-0.40	2.8 21.0	1.68	-0.40	2.1 21.7	1.25	+0.60	2.8 19.6	1.68	-0.40	2.1 22.4	1.25	-0.40	3.5 21.7	0.69
3	-0.50	2.8 22.4	1.68	-0.50	4.8 42.7	0.34	+1.10	2.8 19.6	1.68	+1.00	2.8 21.0	1.68	-0.40	2.8 21.0	1.68	-0.40	2.1 21.7	1.25	+1.70	2.8 22.4	1.68	-0.40	2.8 21.7	1.68	-0.40	4.2 21.0	0.89
6	-0.50	2.8 22.4	1.68	-0.50	4.8 42.7	0.34	+1.30	2.8 19.6	1.68	+1.00	2.8 21.0	1.68	-0.40	2.8 21.0	1.68	-0.40	2.1 21.7	1.25	+1.80	2.8 22.4	1.68	-0.40	3.5 19.6	2.08	-0.40	4.2 21.0	0.89
12	-0.50	2.8 23.1	1.68	-0.40	4.8 42.7	0.34	+1.30	2.8 19.6	1.68	+1.50	2.8 21.0	1.68	-0.60	3.5 23.1	2.08	-0.40	2.8 22.4	1.68	+1.70	2.8 22.4	1.68	-0.40	4.2 21.0	2.50	-0.40	4.2 24.5	0.89
Control.....	-0.50	3.5 23.1	2.00	-0.50	4.3 42.0	0.47	-0.10	3.5 21.7	2.00	-0.60	3.5 23.1	2.00	-0.60	3.5 23.1	2.00	-0.60	3.5 23.1	2.00	-0.60	3.5 23.1	2.00	-0.60	3.5 23.1	2.00	-0.60	7.0 21.0	1.41
Culture B....	-0.60	4.2 23.4	2.40	-0.60	4.8 42.0	0.47	+1.40	3.5 21.7	2.00	+0.90	3.5 22.4	2.00	-0.50	3.5 21.7	2.00	-0.40	3.5 21.7	2.00	-0.40	3.5 22.4	2.00	-0.60	4.2 21.7	2.40	-0.60	7.0 22.4	1.41
3	-0.60	4.2 23.4	2.40	-0.60	4.8 42.0	0.53	-1.70	3.5 21.7	2.00	+1.00	4.2 22.4	2.40	-0.60	3.5 21.7	2.00	-0.40	3.5 21.7	2.00	-0.40	3.5 22.4	2.00	-0.60	4.2 21.7	2.40	-0.60	7.0 21.0	1.41
6	-0.60	4.2 23.5	2.40	-0.60	4.8 42.7	0.53	-1.70	3.5 21.7	2.00	+1.00	4.2 22.4	2.40	-0.60	3.5 21.7	2.00	-0.40	3.5 21.7	2.00	-0.40	3.5 22.4	2.00	-0.50	4.2 21.7	2.40	-0.50	7.0 21.7	1.41
13	-0.60	4.2 23.8	2.40	-0.60	5.6 44.1	0.63	-1.60	3.5 23.1	2.00	+1.60	4.2 23.8	2.40	-0.60	4.3 22.4	2.40	-0.40	3.5 23.1	2.40	-0.40	4.3 23.8	2.40	-0.40	4.2 21.7	2.40	-0.40	6.3 21.7	1.27
21	-0.60	4.2 23.8	2.40	-0.60	5.6 44.1	0.63	+1.70	3.5 23.1	2.00	+1.10	4.2 23.8	2.40	-0.80	4.3 23.1	2.40	-0.40	4.9 24.5	2.80	+1.60	4.2 24.5	2.40	-0.40	4.2 23.1	2.40	-0.40	6.3 21.7	1.27

Reaction: — = alkaline to neutral red; + = acid to neutral red; c = normal acid or alkali per 100 c.c. of medium.

Ammonia and amino nitrogen expressed in milligrams per 100 c.c. of culture medium.

Control indicates initial composition of medium. Net results may be obtained by subtracting the control from the day's analysis.

Amino nitrogen is corrected in each instance for ammonia.

*Origin of Cultures.*—*B. tertius*, Culture A, was obtained from Dr. Holman, who brought it from Paris.

*B. tertius*, Culture B, was isolated from the fecal contents of a man exhibiting an overgrowth of Welch bacilli in the alimentary canal.

#### DISCUSSION

Chemically, *B. tertius* is one of the more inert varieties of anaerobic bacilli. The nitrogenous changes induced by the organism are quantitatively very similar to those of *B. fallax*.<sup>6</sup> In mediums containing no utilizable carbohydrate, the nitrogenous changes even on prolonged incubation are minimal. Neither the ammonia nor the amino nitrogen exhibits a deviation from the uninoculated controls which is in excess of the probable error of the method, namely, about 2 mg. above or below the controls. The nitrogenous changes in plain broth, glycerol, and starch mediums (which have the same nitrogenous content) are almost identical, as might confidently be expected, as the glycerol and starch are not utilizable as sources of energy. In gelatin also the nitrogenous changes are practically negligible. Attempts to demonstrate a soluble, proteolytic enzyme in gelatin cultures of *Bacillus tertius* were uniformly negative. The gelatin remained firm even after three weeks' incubation. Glucose, lactose, saccharose and mannitol are fermented with the production of some gas, and a moderate but distinct increase in titratable acidity. The decomposition of the carbohydrates is slow, the evolution of gas is very moderate, and the accumulation of acid products of fermentation is very deliberate.

Culture B differed from culture A in that its action on milk was very slow. After a few weeks only a few cubic centimeters of gas were formed, and the direct examination of the milk cultures by the method of Gram staining revealed only a few bacilli. It would appear that this particular organism failed to develop with even moderate luxuriance in the milk medium.

#### SUMMARY

*B. tertius* is an anaerobic bacillus, characterized morphologically by the stainable properties of immature spores. The mature spores are terminal, and distinctly oval. This is a point of differentiation from *Bacillus tetani*, with which the organism might be confused on purely morphologic grounds.



Culturally, the organism is relatively inert. Its action on protein (or protein derivatives) is minimal, in which respect it suggests *B. fallax* strongly.

In mediums containing utilizable carbohydrates there is a very moderate evolution of gas, chiefly  $H_2$  and  $CO_2$ , and a coincident increase in titratable acidity. Acid and gas are produced from glucose, lactose, saccharose, and the hexose alcohol, mannitol. In the latter respect, that is, the gaseous fermentation of mannitol, *B. tertius* is quite distinctive among the members of the carbohydrophilic anaerobic group, to which it belongs.

The inability of *B. tertius* to utilize either glycerol or starch for energy, together with its ability to ferment mannitol, would appear to be distinguishing features, definitely differentiating it culturally from *B. welchii*, *Vibrio septique* or *B. fallax*. Its negative effect on nitrogenous substances is an additional distinguishing characteristic.

## BACILLUS TETANI

### STUDY XLVIII

*B. tetani* is the most widely known of the anaerobic bacilli. Indeed, since Carle and Rattoni<sup>1</sup> inoculated a rabbit with pus from a human case of the disease and reproduced the essential clinical features of the disease in 1884, the occasional case of tetanus has been widely heralded even in the public press. The dread of the wound inflicted with a "rusty nail" has become a public heritage which perhaps has its origin in the experiments of Nicolaier,<sup>2</sup> who induced lesions in laboratory animals by the subcutaneous injection of garden soil. Kitasato's<sup>3</sup> great discovery of the toxin of the tetanus bacillus and the preparation of a specific antitoxin on a practical scale completed the really significant available information of the growth of the organism and the nature of its products up to the Great War.

The mechanism of the production of tetanus toxin is of no concern in the present discussion. An additional discovery of importance, produced as a result of the intensive study of tetanus bacilli derived from wounds of warfare is that of Tulloch,<sup>4</sup> who described four types of

<sup>1</sup> Gior. d. r. Accad. di med. di Torino, 1884, No. 3.

<sup>2</sup> Deutsch. med. Wchnschr., 1884, 10, p. 842.

<sup>3</sup> Deutsch. med. Wchnschr., 1889, 15, p. 635; Ztschr. f. Hyg., u. Infektionskr., 1889, 7, p. 225.

<sup>4</sup> Jour. Hygiene, 1919, 18, p. 103.

tetanus bacilli, each of which appears to be a serological entity. In this respect the tetanus bacillus is reminiscent of the four serological types of the meningococcus and the pneumococcus. Tetanus antitoxin, however, unlike the meningococcus immune serums, is qualitatively, and for practical purposes quantitatively, a specific neutralizing agent for the toxin of any of the four types.<sup>4</sup>

As the principal cultural substance formed by the tetanus bacillus as a result of its growth is the soluble, and tremendously potent toxin, it is not unnatural that the identification of the organism in the past has been restricted practically to the determination of this point. The cultural identification has been largely overlooked, or at best imperfectly scrutinized. The toxin, furthermore, is said not to be materially reduced in potency when it is developed by cultures of tetanus bacilli contaminated with alien organisms. For this reason, as the usual interest surrounding *B. tetani* is the production of toxin for purposes of immunization, few attempts at the cultural study of the organism, with adequate methods, are on record.

The commonly accepted characteristics of *B. tetani* suggest strongly a proteolytic aspect, and endowed with fermentative powers as well. Indeed, the directions for cultivating tetanus bacilli for toxin production stress the formation of considerable gas during the earlier stages of the process, and the gradual development of a foul odor later, when toxin begins to accumulate.

Achalme<sup>5</sup> appears to have been the first, or at least one of the earliest, investigators to call attention to the fact that the tetanus bacillus fermented no carbohydrates. This view apparently is accepted by the Medical Research Committee.<sup>6</sup> The latter, however, state that gelatin is liquefied, while coagulated serum exhibits little or no liquefaction, suggesting at least mild proteolytic powers. In this respect the Medical Research Committee would apparently place the Welch bacillus and *Vibrio septique* in a group exhibiting both saccharolytic and proteolytic activities, the former predominating. Undoubtedly, the fluidification of gelatin, exhibited by both the Welch organism and *Vibrio septique*, would be a determining factor in such a classification. Chemical analyses of gelatin cultures of the organisms inciting gas gangrene<sup>7, 8</sup> have shown that the quantitative nitrogenous changes in

<sup>4</sup> Ann. Inst. Past., 1902, 16, p. 633.

<sup>5</sup> Report on Anaerobic Infections of Wounds, 1919.

<sup>7</sup> Wolf: Jour. Path. & Bacteriol., 1917, 21, p. 386; *ibid.*, 1918, 22, p. 115.

<sup>8</sup> Kendall, Day and Walker: Jour. Infect. Dis., Studies XLIV and XLV, 1922, 30, pp. 141 and 155.

TABLE 1  
BACILLUS TETANI

Day	Platin			Gelatin			Glucose			Mannitol			Glycerol			Lactose			Saccharose			Starch			Milk		
	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen
Control.....	-1.00	14.7 32.9	5.00	-0.40	24.5 39.9	3.21	-0.60	14.0 31.5	4.76	-0.90	14.7 32.2	5.00	-0.90	14.7 32.7	5.00	-0.80	14.0 30.8	4.76	-1.00	14.0 32.2	4.76	-0.90	14.0 32.9	4.76	+1.70	5.6 21.0	1.14
Culture A....	-0.80	16.8 25.9	5.72	-0.30	29.4 35.0	3.87	-0.40	15.4 30.3	5.22	-0.70	15.4 32.6	5.22	-0.70	14.7 25.2	5.00	-0.60	15.4 33.8	5.22	-0.80	15.4 32.2	5.22	-0.60	15.4 32.9	5.22	+1.70	7.7 18.9	1.57
3	-0.80	20.3 26.6	6.90	-0.30	32.2 35.7	4.22	-0.20	18.2 21.7	6.19	-0.60	20.3 23.1	6.19	-0.20	18.2 21.7	6.19	-0.50	21.7 30.3	7.38	-0.70	20.3 23.1	6.90	-0.60	21.7 30.3	7.38	+1.80	8.4 18.2	1.71
6	-0.70	25.2 28.7	8.57	-0.20	30.8 39.2	4.04	-0.10	20.3 22.4	6.90	-0.60	23.8 23.8	8.10	-0.10	20.3 25.2	6.90	-0.50	22.4 33.8	7.62	-0.70	25.2 28.7	8.57	-0.60	26.6 27.3	9.06	+1.80	8.4 18.2	1.71
13	-0.60	28.0 32.9	9.53	-0.10	39.9 38.5	5.23	+0.10	21.7 23.8	7.88	-0.40	25.2 25.2	8.57	+0.10	23.8 25.2	8.10	-0.30	25.7 25.7	9.76	-0.50	26.6 28.0	9.01	-0.50	30.8 27.3	10.4	+1.80	9.8 19.6	2.00
21	-0.60	28.0 31.5	9.53	-0.10	42.7 37.1	5.60	+0.20	22.4 27.3	7.62	-0.50	28.0 30.8	9.53	+0.20	27.3 30.8	9.30	-0.40	29.4 25.0	10.0	-0.60	30.1 31.5	10.2	-0.60	32.2 35.7	10.9	+1.90	11.9 21.7	2.43
Control.....	-0.60	3.5 24.5	1.84	-0.70	4.2 46.2	0.44	-0.10	4.2 21.0	2.22	-0.60	3.5 24.5	1.84	-0.60	3.5 24.5	1.84	-0.50	3.5 24.5	1.84	-0.60	3.5 24.5	1.84	-0.60	3.5 24.5	1.84	+1.40	7.0 21.0	1.43
Culture B....	-0.40	7.7 19.6	4.07	-0.60	7.0 39.2	0.73	+0.20	4.9 16.1	2.59	-0.40	7.7 19.6	4.07	-0.30	4.2 18.2	2.22	-0.20	7.7 19.6	4.07	-0.30	6.3 20.3	3.33	-0.30	7.7 19.6	4.07	+1.40	9.1 18.9	1.86
3	-0.30	12.6 21.7	6.63	-0.40	9.8 42.0	1.03	+0.30	9.8 16.1	5.18	-0.30	10.5 21.7	5.56	-0.40	10.5 19.6	5.56	-0.20	10.5 21.0	5.56	-0.20	9.8 21.0	3.18	-0.40	11.2 21.7	5.93	+1.40	9.1 18.9	1.86
6	-0.20	14.7 24.5	7.78	-0.30	14.7 46.2	1.54	+0.50	10.5 18.2	5.56	-0.30	11.9 25.2	5.93	-0.30	11.9 23.1	5.93	-0.10	14.7 26.6	7.78	-0.20	12.6 23.8	6.66	-0.20	13.3 25.9	7.03	+1.40	9.8 20.3	2.00
13	-0.20	18.2 24.5	9.63	-0.40	21.7 44.1	2.28	+0.50	11.9 17.5	6.30	-0.20	14.7 28.7	7.78	-0.30	16.1 26.6	8.52	-0.10	15.4 25.9	8.15	-0.20	15.4 25.9	8.15	-0.20	15.4 25.9	8.15	+1.50	9.8 21.0	2.00
21	.....	.....	.....	-0.30	23.8 42.0	2.50	+0.60	13.3 17.5	7.03	-0.20	17.5 28.0	9.26	-0.20	16.1 26.6	8.52	.....	.....	.....	-0.10	16.1 26.6	8.62	-0.30	15.4 25.0	8.15	+1.50	10.5 21.0	2.14
Control.....	-0.70	2.5 26.6	1.43	-1.00	4.2 42.0	0.45	-0.10	2.8 22.4	1.43	-0.70	3.5 25.9	1.78	-0.70	2.8 26.6	1.43	-0.60	3.5 25.9	1.78	-0.70	3.5 25.9	1.78	-0.70	3.5 25.9	1.78	+1.00	5.6 22.4	1.12
Culture C....	-0.70	4.9 24.5	2.50	-0.80	6.3 32.9	0.68	+0.10	4.9 23.1	2.50	-0.50	7.0 18.9	3.63	-0.40	4.9 26.6	2.50	-0.60	6.3 23.1	3.21	-0.50	9.1 18.2	4.61	-0.50	5.6 18.2	2.85	+1.20	6.3 18.2	1.29
3	-0.50	7.0 18.2	3.63	-0.70	10.5 32.9	1.13	+0.20	6.3 24.5	3.21	-0.50	9.8 17.5	5.00	-0.30	9.1 22.4	4.61	-0.40	8.4 20.3	4.28	-0.50	11.4 18.9	5.72	-0.50	9.8 18.9	5.00	+1.40	7.7 21.7	1.55
6	-0.40	9.1 17.5	4.64	-0.70	16.1 32.9	1.74	+0.40	7.0 24.5	3.63	-0.30	11.2 23.8	5.72	-0.20	12.6 21.7	6.42	-0.40	9.8 17.5	5.00	-0.50	12.6 19.6	6.42	-0.40	12.6 19.6	6.42	+1.50	10.5 22.4	2.11
13	-0.40	12.6 16.1	6.42	-0.60	19.6 32.9	2.12	+0.40	9.8 21.0	5.00	-0.10	13.3 23.8	6.78	-0.20	13.3 22.4	6.78	-0.30	11.2 19.6	5.72	-0.30	14.7 22.4	7.50	-0.30	15.4 21.7	7.86	+1.70	11.9 23.1	2.39

Reaction: alkaline to neutral red; + normal acid to neutral red; - normal acid to neutral red; + normal acid to neutral red; - normal acid to neutral red.

Ammonia and amino nitrogen expressed in milligrams per 100 c.c. of culture medium.

Control indicates initial composition of medium. Net results may be obtained by subtracting the control from the day's analysis.

Amino nitrogen is corrected in each instance for ammonia.

gelatin mediums are of very small magnitude, and wholly different from those characteristic of bacteria which cause deep-seated, true liquefactions in these mediums.<sup>9</sup>

It should be stated that those bacteria which produce powerful, soluble toxins incidentally to the utilization of protein for energy, as the diphtheria bacillus, are characterized by their slight action on protein, as shown by a study of the nitrogenous metabolism of such cultures.<sup>10</sup> So far as available information indicates, true toxin formation appears to be incompatible with marked proteolysis.

*Origin of Cultures.*—*B. tetani* A—A strain used extensively in the United States to produce tetanus toxin.

*B. tetani* B—An old culture obtained from Dr. Theobald Smith.

*B. tetani* C—Isolated from garden soil.

#### DISCUSSION

The nitrogenous changes are somewhat more marked than those characteristic of the carbohydrophilic organisms studied previously—*B. welchii*, *B. fallax*, and *Vibrio septique*. In each strain there is a moderate production of ammonia, amounting to approximately 20-30 mg. for each 100 c.c. of medium. These figures are of the same order of magnitude as those characteristic for the diphtheria bacillus for an equal period of incubation. The amino nitrogen shows a small but definite decrease in each instance. This suggests, but of course does not prove, that the tetanus bacillus utilizes that portion of the nitrogenous constituents of the various mediums which contain  $\text{NH}_2$  groups capable of uniting with formaldehyde for at least a part of their energy. It will be remembered that Buchner<sup>11</sup> claimed to have demonstrated toxin formation to a limited extent in a modified Uschinsky medium, which contains no protein whatsoever. Asparagin was the only organized source of nitrogen available for the organism in his experiments. It should be stated that Brieger<sup>12</sup> failed to confirm Buchner's observations.

Some gas was formed in all the mediums studied. The amount was small and the rate of accumulation was small. The gas formed was not analyzed. It was as great in amount in plain, nutrient broth as

<sup>9</sup> Kendall and Walker: *Ibid.*, 1915, 17, p. 442.

<sup>10</sup> Kendall and Farmer: *Jour. Biol. Chem.*, 1912, 12, p. 13. Kendall, Day and Walker: *Jour. Am. Chem. Soc.*, 1913, 35, p. 1201.

<sup>11</sup> *München. med. Wehnschr.*, 1893, 40, 449.

<sup>12</sup> *Ztschr. f. Hyg. u. Infektionskr.*, 1895, 19, p. 101.

in gelatin, glucose broth or any of the mediums studied. Coincidentally, there was a slow rise in the titratable acidity of the mediums.

Gelatin was not softened, even after an incubation of nearly three weeks, and the nitrogenous changes induced by the organisms are quantitatively small and not suggestive of proteolytic tendencies.

The addition of the ordinary carbohydrates—glucose, lactose, saccharose, mannitol, or starch—failed to increase materially either the luxuriance of growth or to augment in a noteworthy manner the rate or amount of gas production. The changes observed in titratable acidity in carbohydrate mediums above those of corresponding nonsaccharine mediums, furthermore, with respect to titratable acidity or otherwise, were quantitatively undetectable.

#### SUMMARY

The strains of tetanus bacillus discussed herein produced a soluble toxin, a very small amount of which, amounting to 0.05 c c, would kill white mice. No attempt was made to determine the minimal lethal dose, however. The sole purpose of the mouse inoculation was to establish the presence of a soluble toxin which would induce qualitatively a typical fatal effect on the animal.

Morphologically the organisms were perfectly typical. Chemically they were relatively inert.

The changes induced in the nitrogenous constituents of the ordinary cultural mediums were limited. There was a small, but definite and gradual, accumulation of ammonia, which was quantitatively the same, irrespective of the non-nitrogenous constituents. Simultaneously, and at nearly the same rate, there was a diminution in the amount of "amino-nitrogen," as shown by the method of formol titration.<sup>13</sup>

Gelatin was not softened, and there was no visible change in the appearance of milk. A gradual increase in titratable acidity was demonstrated in each medium, and coincidentally a slow and limited evolution of gas occurred. Presumably the gas was derived from some of the protein constituents of the mediums.

Carbohydrates were not decomposed in a measurable degree.

The cultures identified and studied as *Bacillus tetani* are not carbohydrophilic. They are feebly proteolytic. Chemically, the organisms are characterized by their relative inertness.

<sup>13</sup> Sørensen: *Ztschr. physiol. Chem.*, 1910, 64, p. 120.

## BACILLUS PSEUDOTETANI

## STUDY XLIX

The organism discussed here as pseudotetanus appears to be identical with *B. tetanomorphus* of the Medical Research Committee.<sup>1</sup> They in turn establish identity with the *B. pseudotetani* of McIntosh and Feldes.<sup>2</sup> The organism described in 1898 by Tavel<sup>3</sup> as a pseudotetanus bacillus is very probably the earliest description which is in reasonable agreement with the organism under discussion. It is possible that Debono's organism, *B. anaerobicus-alcaligenes*,<sup>4</sup> Fleming's bacillus,<sup>5</sup> and the bacillus of Adamson and Cutler<sup>6</sup> may be identical or at least closely related forms.

The microbe, as the name suggests, exhibits the morphology of *Bacillus tetani*. It differs from *B. tetani*, however, in that no soluble toxin is demonstrable in cultures in any medium. Aside from the resemblance of *B. pseudotetani* to the true toxin producing *B. tetani*, it has no distinctive characteristics. The strain studied as *B. pseudotetani* was obtained by Dr. Holman in England.

It exhibits the characteristics described and agrees with the generally accepted description of the organism which it is supposed to represent in being practically without action on gelatin or other protein, and in inducing a gaseous fermentation in glucose. In addition a few gas bubbles evolve in a plain, cooked meat medium, which becomes somewhat red in color. A small amount of gas is evolved in all mediums. The volume, however, except in glucose cultures, is detectable but insignificant as indicating a true saccharine fermentation.

The nitrogenous changes induced in the ordinary mediums by *B. pseudotetani* are of moderate intensity. There is a slight, steady increase in the ammonia content of the mediums, amounting finally, after 2 weeks' incubation, to about 30 mg. per 100 cc of culture medium above that of the controls. Like *B. tetani*,<sup>7</sup> the amino-nitrogen decreases in amount as the ammonia increases. This is especially the case during the earlier days of incubation when the birth-rate of the organisms is very high. Later, when the products of metabolism slow

<sup>1</sup> Report 39, 1919.

<sup>2</sup> Report 12, 1917.

<sup>3</sup> Centralbl. f. Bakteriöl., 1898, 23, p. 538.

<sup>4</sup> Centralbl. f. Bakteriöl., I, O., 1912, 62, p. 229.

<sup>5</sup> Lancet, 1915, 2, p. 376.

<sup>6</sup> Lancet, 1917, 1, p. 688.

<sup>7</sup> Kendall, Day and Walker: Study XLVIII, Jour. Infect. Dis., 1922, 30, p. 170.

TABLE 1  
BACILLUS PSEUDOTETANI

Day	Plain			Gelatin			Glucose			Mannitol			Glycerol			Lactose			Saccharose			Starch			Milk			
	Reaction	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	
Control.....	-0.90	14.7	35.0	5.53	-0.60	16.1	35.7	2.53	-0.50	14.0	32.2	5.26	-0.90	14.0	33.6	5.26	-0.90	14.0	33.6	5.26	-0.90	14.0	34.3	5.23	-0.90	14.7	35.0	5.53
Culture A....	+0.20	16.8	25.2	6.82	+0.10	14.7	36.4	2.81	+0.50	14.7	27.3	5.53	+0.30	16.8	23.8	6.82	+0.60	16.1	23.1	6.06	+0.90	14.7	28.7	6.82	+0.50	16.8	25.2	6.82
1	+0.60	23.1	27.3	8.68	+0.20	15.4	37.1	2.42	+3.80	14.0	23.8	5.26	+0.60	16.1	25.9	6.06	+0.70	21.7	20.3	8.16	+0.80	20.3	23.8	7.63	+0.70	22.4	25.2	8.42
6	+0.70	25.2	29.4	9.43	+0.40	19.6	35.7	3.07	+3.90	14.0	26.6	5.26	+0.70	22.4	28.0	8.43	+2.60	21.0	28.0	7.90	+0.70	23.1	25.9	8.68	+0.70	23.1	28.7	8.68
13	+0.70	28.0	33.6	10.5	+0.50	21.0	37.1	3.29	+3.10	16.1	25.9	6.06	+0.60	26.6	29.4	10.0	+2.40	24.5	28.7	9.23	+0.80	26.6	32.2	10.9	+0.60	26.6	29.4	10.0
21	+0.70	31.5	32.9	11.8	+0.60	21.7	40.6	3.41	+2.70	17.5	34.3	6.58	+0.60	23.8	32.2	8.36	+2.00	25.2	36.4	9.48	+0.90	30.1	33.6	11.3	+0.90	28.7	31.5	10.7

Reaction: — alkaline to neutral red; + = acid to neutral red; c.c. normal acid or alkali per 100 c.c. of medium.  
Ammonia and amino nitrogen expressed in milligrams per 100 c.c. of culture medium.  
Control indicates initial composition of medium. Net results may be obtained by subtracting the control from the day's analysis.  
Amino nitrogen is corrected in each instance for ammonia.



up the reproductive process, the amino-nitrogen tends to increase somewhat, approaching finally in amount that of the uninoculated controls.

The total nitrogenous change, however, is so slight in comparison to the total nitrogenous content of the mediums that the pseudotetanus bacillus can hardly be classed as one of the proteophilic group.

Gelatin is not softened, even after prolonged incubation, and the nitrogenous changes in this medium are scarcely greater than those of plain, nutrient broth in which the amount of protein nitrogen is scarcely 20% as great. No evidences of a soluble, proteolytic enzyme were obtained. The only carbohydrate that was fermented was glucose. The analytic table shows the sharp rise in titratable acidity associated with the fermentation of this hexose sugar. A decided increase in acidity with an evolution of some gas was observed in glycerol. Glycerol, however, did not stimulate the rate or volume of gas formation in a marked degree, as did glucose. Indeed, the amount of gas observed in glycerol cultures was not greatly in excess of that formed in the other mediums not containing glucose.

*B. pseudotetani*, therefore, appears to be an anaerobic bacillus, exhibiting the morphology of *B. tetani*, but devoid of toxicogenic powers. The organism is culturally quite inert. It is without noteworthy proteolytic powers, and it is nearly devoid of fermentative powers, as well. Glucose (and maltose) appear to be the only carbohydrates which *B. pseudotetani* can utilize for energy. The principal significance of the microbe lies in its remarkable resemblance to *B. tetani*, and its possibility of occurrence in associations in which *B. tetani* would be sought for as a matter of routine.

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## BACILLUS BOTULINUS

### STUDY L

*Bacillus botulinus* does not appear to have been isolated from infected wounds of warfare; as a majority of strains grow rather poorly at body temperature, it is not improbable that the microbe may be disregarded as an incitant of gunshot wound infections. The organism is a formidable toxin producer, although leading apparently a saprophytic existence; therefore it is to be regarded as one of the dangerous anaerobic bacteria. *B. botulinus*, as von Ermengem, its discoverer, showed many years ago,<sup>1</sup> forms a soluble toxin under appropriate con-

<sup>1</sup> Centralbl. f. Bakteriöl., 1896, 19, p. 442; Ztschr. f. Hyg. u. Infektionskr., 1897, 26, p. 1.

TABLE 1

## BACILLUS BOTULINUS

Day	Plain			Gelatin			Glucose			Mannitol			Glycerol			Lactose			Saccharose			Starch			Milk		
	Reaction	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Amino Nitrogen	Percentage Ammonia to Total Nitrogen
Control.....	-0.60	5.619.6	3.33	-0.60	7.044.1	0.77	-0.30	5.616.8	3.33	-0.60	5.619.6	3.33	-0.50	5.619.6	3.33	-0.60	5.619.6	3.33	-0.60	5.619.6	3.33	-0.60	5.619.6	3.33	-0.60	5.619.6	3.33
Culture A....	-0.60	5.622.4	2.33	-0.30	5.638.5	0.63	+0.30	7.045.5	4.16	+0.30	7.212.0	4.58	-0.10	5.619.6	3.33	-0.60	9.120.3	5.41	-0.60	6.322.4	3.75	-0.60	5.619.6	3.33	-0.60	5.619.6	3.33
2	-0.60	5.617.7	12.5	-0.10	13.422.0	1.69	+1.30	10.352.9	6.25	-0.30	15.216.8	10.8	+0.60	9.117.5	5.31	-0.40	21.013.2	12.5	-0.30	18.218.9	10.8	-0.60	5.619.6	3.33	-0.60	5.619.6	3.33
6	-0.60	5.625.9	16.8	+0.20	33.653.5	3.68	+1.30	12.628.7	7.30	-0.20	17.322.0	16.2	+1.10	14.020.3	8.33	-0.30	25.233.8	15.9	-0.20	30.120.3	17.7	-0.60	5.619.6	3.33	-0.60	5.619.6	3.33
13	-0.60	5.627.3	19.5	+0.30	35.538.5	4.23	+2.10	14.128.7	8.75	-0.10	15.421.7	19.5	+1.30	13.421.7	7.50	-0.10	32.824.5	18.5	-0.20	31.527.8	20.8	-0.60	5.619.6	3.33	-0.60	5.619.6	3.33
21	-0.60	5.625.9	20.0	+0.70	37.156.0	4.08	+2.10	14.026.6	8.33	-0.30	31.124.5	22.1	+1.30	12.623.1	7.50	-0.10	37.123.8	22.1	-0.30	34.322.4	20.4	-0.60	5.619.6	3.33	-0.60	5.619.6	3.33
Control.....	-0.60	5.619.6	3.33	-0.60	7.044.1	0.77	-0.30	5.616.8	3.33	-0.60	5.619.6	3.33	-0.50	5.619.6	3.33	-0.60	5.619.6	3.33	-0.60	5.619.6	3.33	-0.60	5.619.6	3.33	-0.60	5.619.6	3.33
Culture B....	-0.60	6.336.4	0.69	-0.50	6.336.4	0.69	+0.60	3.517.5	2.08	-0.60	7.720.3	4.58	-0.50	7.019.6	4.16	-0.50	4.219.6	2.50	-0.60	5.619.6	3.33	-0.60	5.619.6	3.33	-0.60	5.619.6	3.33
3	-0.60	6.321.0	3.75	-0.30	15.442.0	1.69	+1.40	8.420.3	5.00	-0.30	17.517.5	10.4	+1.10	6.320.3	3.75	-0.40	16.821.7	10.0	-0.40	14.021.7	8.33	-0.60	5.619.6	3.33	-0.60	5.619.6	3.33
6	-0.60	6.330.1	12.0	-0.10	28.049.7	3.07	+1.50	11.222.4	6.66	-0.10	31.521.0	18.7	+1.20	6.319.6	5.41	-0.10	23.520.3	24.0	-0.30	21.720.3	12.9	-0.60	5.619.6	3.33	-0.60	5.619.6	3.33
13	-0.60	6.331.2	16.2	+0.20	34.556.0	3.77	+2.20	13.324.5	7.92	-0.10	35.721.7	21.2	+1.50	12.621.0	7.50	-0.00	33.621.7	20.8	-0.00	24.523.1	14.5	-0.60	5.619.6	3.33	-0.60	5.619.6	3.33
20	-0.60	6.359.9	20.8	+0.40	39.356.7	3.21	+2.30	14.725.2	8.75	-0.10	37.121.7	22.1	+1.60	13.322.4	7.50	+0.10	35.020.3	20.8	-0.30	29.625.2	17.6	-0.60	5.619.6	3.33	-0.60	5.619.6	3.33
Control.....	-0.60	3.518.9	1.92	-0.50	4.239.2	0.47	-0.10	3.517.5	1.92	-0.60	3.518.9	1.92	-0.60	3.518.9	1.92	-0.50	3.518.9	1.92	-0.60	3.518.9	1.92	-0.60	3.518.9	1.92	-0.60	3.518.9	1.92
Culture C....	-0.30	8.417.5	4.83	-0.30	4.933.6	0.55	+0.30	4.218.2	2.30	-0.60	6.314.0	3.46	-0.10	5.622.4	1.92	-0.40	8.421.7	4.83	-0.50	5.618.9	3.07	-0.60	3.518.9	1.92	-0.60	3.518.9	1.92
3	-0.30	8.417.5	4.83	-0.10	16.139.9	1.81	+1.10	7.721.7	4.23	-0.30	12.619.6	6.92	+1.10	9.121.7	5.00	-0.10	15.420.3	8.46	-0.30	17.521.7	9.69	-0.60	3.518.9	1.92	-0.60	3.518.9	1.92
6	-0.10	18.223.1	10.0	-0.10	25.247.6	2.83	+1.60	14.025.2	7.69	-0.10	23.420.3	16.1	+1.60	11.323.1	6.16	-0.10	24.524.5	13.4	-0.10	25.247.6	2.83	-0.60	3.518.9	1.92	-0.60	3.518.9	1.92
12	-0.10	24.526.6	13.4	+0.20	31.549.7	3.54	+2.20	16.128.0	8.85	-0.20	35.723.1	19.6	+1.70	13.423.2	8.46	-0.10	31.520.3	17.3	+0.10	30.827.3	16.9	-0.60	3.518.9	1.92	-0.60	3.518.9	1.92
Control.....	-0.60	5.619.6	3.33	-0.60	7.044.1	0.77	-0.30	5.616.8	3.33	-0.60	5.619.6	3.33	-0.50	5.619.6	3.33	-0.60	5.619.6	3.33	-0.60	5.616.8	3.33	-0.60	5.619.6	3.33	-0.60	5.619.6	3.33
Culture D....	-0.50	7.717.5	4.53	-0.50	11.234.3	1.23	-1.10	4.924.5	2.91	-0.50	8.419.6	5.00	-0.40	7.015.4	4.16	-0.40	10.512.6	6.25	-0.30	9.118.2	5.42	-0.60	5.619.6	3.33	-0.60	5.619.6	3.33
3	-0.40	15.424.1	9.16	-0.40	20.344.1	2.23	-1.40	9.819.6	5.81	-0.30	17.517.5	10.4	-0.10	8.411.2	5.00	-0.30	16.819.6	10.0	-0.10	13.316.8	7.92	-0.60	5.619.6	3.33	-0.60	5.619.6	3.33
6	-0.10	23.223.1	19.1	-0.10	26.556.0	2.92	-1.80	9.819.6	5.81	-0.10	24.518.2	14.5	+1.00	10.621.7	6.25	-0.10	22.423.8	13.3	+0.10	19.621.7	11.6	-0.60	5.619.6	3.33	-0.60	5.619.6	3.33
13	-0.30	33.623.8	20.0	+0.10	34.559.5	3.77	+2.30	16.121.7	9.06	-0.20	35.723.1	19.6	+1.60	15.423.1	9.16	-0.10	23.222.4	15.0	+0.20	22.423.8	13.3	-0.60	5.619.6	3.33	-0.60	5.619.6	3.33
20	-0.10	35.023.8	20.8	+0.20	35.557.4	4.23	+2.00	16.816.8	10.0	+0.10	37.124.5	23.4	+1.70	15.423.1	9.16	-0.10	30.823.8	18.3	+0.20	28.723.3	17.0	-0.60	5.619.6	3.33	-0.60	5.619.6	3.33
Control.....	-0.70	11.221.0	5.16	-0.70	11.236.4	1.63	-0.80	17.527.3	8.06	-0.60	17.521.0	8.05	-0.60	17.524.5	8.05	-0.80	17.527.3	8.05	-0.70	17.527.3	8.05	-0.60	17.527.3	8.05	-0.60	17.527.3	8.05
Culture E....	-0.50	14.019.6	6.45	-0.60	14.036.4	2.04	-1.80	19.924.5	8.72	-0.50	17.518.9	8.05	-0.10	16.830.8	7.74	-0.50	15.417.5	7.10	-0.50	16.122.4	7.42	-0.60	17.527.3	8.05	-0.60	17.527.3	8.05
3	-0.40	19.918.9	8.72	-0.10	17.541.3	2.50	-2.00	19.924.5	8.72	-0.50	22.419.6	10.3	+1.60	17.530.8	8.05	-0.30	16.816.8	10.6	-0.30	17.527.3	8.05	-0.60	17.527.3	8.05	-0.60	17.527.3	8.05
6	-0.20	23.122.4	10.6	+0.20	21.742.7	3.16	+2.30	21.736.6	10.3	-0.30	27.321.0	12.5	+1.70	18.932.9	8.39	-0.30	25.216.8	11.6	-0.30	24.434.3	10.3	-0.60	17.527.3	8.05	-0.60	17.527.3	8.05
9	+0.10	29.429.4	12.5	+0.30	35.744.1	5.20	+2.50	22.336.6	10.3	-0.30	32.222.4	14.5	+1.60	18.933.6	8.72	-0.30	29.421.0	13.5	-0.20	29.421.0	13.5	-0.60	17.527.3	8.05	-0.60	17.527.3	8.05
14	+0.20	29.422.2	13.5	+0.30	41.350.4	6.02	+2.40	21.736.6	10.0	+0.10	31.524.5	24.5	+2.10	19.633.6	9.03	-0.30	30.827.3	14.2	-0.20	21.034.3	9.68	-0.60	17.527.3	8.05	-0.60	17.527.3	8.05
Control.....	-0.70	16.826.6	7.74	-0.70	14.042.0	1.92	-0.40	16.124.5	7.42	-0.60	16.816.8	7.74	-0.60	16.825.2	7.74	-0.50	16.825.2	7.74	-0.70	16.825.2	7.74	-0.60	16.825.2	7.74	-0.60	16.825.2	7.74
Culture F....	-0.50	19.637.1	9.03	-0.50	19.637.1	2.69	+1.80	11.926.6	5.48	-0.50	19.614.0	9.03	-0.20	14.728.0	6.76	-0.30	16.121.7	7.49	-0.30	16.121.7	7.49	-0.60	16.825.2	7.74	-0.60	16.825.2	7.74
3	-0.20	27.928.7	12.5	-0.10	21.011.3	2.84	+2.20	17.530.8	8.06	-0.30	23.415.4	9.95	+1.70	14.728.0	6.76	-0.30	21.012.7	9.03	-0.30	21.012.7	9.03	-0.60	16.825.2	7.74	-0.60	16.825.2	7.74
6	-0.00	29.424.6	13.5	-0.10	27.346.2	3.75	+2.40	18.232.9	8.39	-0.30	25.217.5	11.6	+1.60	19.633.6	9.03	-0.30	25.217.5	11.6	-0.30	25.217.5	11.6	-0.60	16.825.2	7.74	-0.60	16.825.2	7.74
9	+0.20	32.228.7	14.8	+0.30	29.448.3	4.04	+2.50	18.927.3	8.72	-0.30	29.419.6	13.5	+2.10	19.631.5	9.03	-0.30	28.724.5	13.2	-0.20	21.034.3	9.68	-0.60	16.825.2	7.74	-0.60	16.825.2	7.74
14	+0.30	35.030.8	16.1	+0.40	39.248.3	5.38	+2.70	19.628.0	9.03	-0.20	32.221.0	14.8	+2.20	21.731.5	10.0	+0.30	31.523.9	14.5	-0.20	20.328.7	9.36	-0.60	16.825.2	7.74	-0.60	16.825.2	7.74

Reaction: + alkaline to neutral red; - neutral red; + normal pils per 100 c.c. of medium.

Ammonia, and amino nitrogen expressed in milligrams per 100 c.c. of culture medium.

Control indicates initial composition of medium. No results may be obtained by subtracting the control from the day's analysis.

Amino nitrogen is corrected in each instance for ammonia.

ditions which possesses the unique and deadly property of passing the gastro-intestinal tract of man and of animals unharmed. The ingestion of various foods, therefore, has from time to time given rise to single or multiple cases of botulism. As the toxin and not the bacillus is the causative agent of the poisoning, the organism must be sought for, if it is to be recovered, in infected food.<sup>2</sup>

As the organisms described as *B. botulinus* are recognized and identified chiefly through the ability to produce the soluble toxin which resists gastro-intestinal digestion, it is not surprising to find that the published descriptions are somewhat meager and varied. The original description<sup>1</sup> attributed proteolytic properties to *B. botulinus*, and these observations are concurred in up to the present time by many, if not a majority, of observers. Liquefaction of gelatin is described as one of the essential characteristics by the British Medical Committee.<sup>3</sup>

Cultures have been obtained, exhibiting the property of forming a digestion-resistant, soluble toxin, which are without marked proteolytic action on gelatin or other protein. Such cultures may frequently be isolated from supposedly pure strains of *B. botulinus* that induce liquefaction in gelatin after several days' incubation. In such cases the contaminating organism, present at the start in small numbers, gradually becomes prominent and induces a true liquefaction of the medium through the agency of soluble proteolytic enzyme.

Six cultures are studied here. All were purified as described in preceding studies.<sup>4</sup>

*B. botulinus* A was obtained from the Bureau of Animal Industry.

Culture B, from the same source.

Culture C, from a can of pimentoes.

Culture D, from Miss Nevin.

Culture E, from Dr. Karl Meyer.

Culture F, from Dr. Karl Meyer.

#### DISCUSSION

The nitrogenous changes observed in the metabolism of the 6 strains of *B. botulinus*<sup>5</sup> were approximately the same, quantitatively speaking, as those characteristic for cultures of *B. tetani*<sup>6</sup> in so far as

<sup>2</sup> Dickson: Monograph 8, Rockefeller Institute, 1918, contains references to literature to that date.

<sup>3</sup> Report 39, London, 1919.

<sup>4</sup> Kendall, Cook and Ryan: Jour. Infect. Dis., 1921, 29, p. 227.

<sup>5</sup> Toxin that kills guinea-pigs and is resistant to gastro-intestinal digestion was determined in each instance by soaking a crumb of bread in a 10-day culture and feeding it to the animal. The time required to kill varied from approximately 20 to 48 hours.

<sup>6</sup> Kendall, Day and Walker: Study XLVIII, Jour. Infect. Dis., 30, p. 170.

ammonia formation is concerned. The increases observed amount to about 30 or 40 mg. per 100 c c of medium, in those instances in which utilizable carbohydrate is not available as a source of energy. In other words, the strains of *B. botulinus* considered in this series are not proteophilic. Their action for energy on protein or protein derivatives is comparatively slight. This observation is in harmony with the rather general tendency of bacteria to incite moderate changes in protein, even though the observations are carried out for considerable periods of time.

The addition of utilizable carbohydrate to protein mediums reduces materially the amount of ammonia produced. This reduction amounts to 50% in most instances, or even more. Coincidentally, a somewhat greater amount of titratable acidity is noticed. In all mediums, however, there is a moderate but definite increase in titratable acidity, and a slow, slight, but readily detectable evolution of gas takes place. This evolution of gas is more rapid and greater in amount in mediums containing utilizable carbohydrate.

The fermentation reactions of *B. botulinus* are very much in doubt. Some observers claim no carbohydrates are fermented, while others attribute considerable versatility to *B. botulinus* with respect to its ability to utilize carbohydrate for energy. It seems to be generally conceded that glucose is fermented, and polymers of glucose—maltose and starch—also seem to be available sources of non-nitrogenous energy. Glycerol also is said by many observers to be fermented.

The Medical Research Committee<sup>7</sup> states that lactose but not saccharose is fermentable. While information on this point that is wholly satisfactory is not at present available, it may be stated as a somewhat general observation that bacteria which are not parasitic on man or animals frequently fail to utilize lactose, which, it will be remembered, is an animal sugar.

The cultures studied here fermented glucose, maltose and glycerol consistently, although the evolution of gas was rather slow and the general process was relatively sluggish. Starch was slowly decomposed by cultures B and F. Lactose was not visibly attacked. Saccharose was slowly decomposed by cultures F and G. The toxicity of these strains was materially less than that of cultures A, B, C, and D.

The amino-nitrogen changes differed somewhat in type from those observed in cultures of *B. tetani*. The initial decrease of amino

<sup>7</sup> Report 39, 1919.

nitrogen, common to both organisms, was followed by a decided tendency toward an increase above that of the uninoculated controls in the botulinus cultures, as incubation proceeded. The actual change was never great, amounting to less than 40 mg. in 100 c c of medium. This change was less marked in mediums containing utilizable carbohydrate than in purely protein mediums. The significance to be attached to this slight quantitative difference between *B. botulinus* and *B. tetani* cannot be determined in such a limited series of cultures. In this respect, however, the entire field of anaerobic metabolism is yet to be developed.

The nitrogenous changes observed in gelatin and in milk were of the same order of magnitude as those occurring in the simpler protein mediums, such as plain nutrient bouillon. Gelatin was softened after prolonged incubation to a point where it would no longer solidify when placed for several hours at the temperature of the icebox (40 C.), but there was no evidence of a soluble proteolytic enzyme in gelatin cultures, and it appears probable that the action of the organism on protein is relatively limited. It will be remembered that the same phenomenon of softening in gelatin cultures was observed in cultures of the Welch bacillus,<sup>8</sup> and the same explanation is offered for the phenomenon exhibited by *B. botulinus* as that advanced for the corresponding change induced by the Welch bacillus.

#### SUMMARY

The strains of *B. botulinus* studied in this series formed varying amounts of toxin which are resistant to gastro-intestinal digestion.

The fermentation reactions were somewhat variable. Generally speaking, glucose and the polymers of glucose—maltose and starch—appear to be rather more acceptable sources of non-nitrogenous energy than lactose. Saccharose was slowly fermented by two strains. Glycerol was fermented slowly by all.

Some gas was produced even in protein mediums, and the quantitative difference in gas production and gas volume between purely protein and protein-carbohydrate mediums is not great. Considerable caution is required, therefore, in the interpretation of fermentation reactions (gas production) in cultures of *B. botulinus*.

The nitrogenous changes induced by *B. botulinus* in protein mediums are relatively insignificant. The organism cannot be classed as a proteophilic anaerobe. Culturally *B. botulinus* is chemically relatively inert.

<sup>8</sup> Kendall, Day and Walker: Study XLIV, Jour. Infect. Dis., 1922, 30, p. 141.

## BACILLUS BIFERMENTANS

## STUDY LI

*Bacillus bifermentans* (*Bacillus bifermentans-sporogenes*) was described originally by Tissier and Martelly<sup>1</sup> as an anaerobic bacillus exhibiting both carbohydrophilic and proteophilic properties. The organism has been cultured from infected wounds of warfare by Tissier<sup>2</sup> and by Hemple.<sup>3</sup>

The organism fails to exhibit any striking or noteworthy morphologic, serologic or toxicologic features; the changes it induces in culture mediums are quite sharply defined, but not vigorous. One rather definite cultural characteristic is the gradual accumulation of a mucinous deposit in cultures containing utilizable carbohydrate. Small amounts of gas bubbles are slowly evolved from all ordinary cultural mediums. The rate of formation of this gas, and the volume, however, are small. This ability to produce a small amount of gas from cultural mediums, even those containing no carbohydrates, is a rather general one exhibited by nearly all anaerobic bacilli. The origin of the gas, its composition, and its significance, are yet to be determined. The phenomenon is of real importance in connection with gas production from utilizable carbohydrates by anaerobes, however, because the question of fermentability of non-nitrogenous compounds with the liberation of gas is a cultural procedure of diagnostic importance in this group. Elaborate controls must be made to distinguish between the small volume of fundamental gas common to all mediums from the additional evolution of gas from utilizable carbohydrates. Usually the phenomenon is most confusing when determinations are made in small volumes, such as "shake cultures" or Smith fermentation tubes. When larger volumes of mediums—100 c c or more—are studied, the fundamental gas volume becomes less difficult of evaluation because of the larger proportionate accumulation of gaseous products from the decomposition of significant amounts of sugars or their derivatives.

The fermentation reactions accredited to *B. bifermentans* comprise the gaseous fermentation of glucose, levulose, and maltose.<sup>4</sup> From these acid is produced, and gas is slowly evolved. Tissier and Martelly<sup>1</sup>

<sup>1</sup> Ann. Inst. Past., 1902, 16, p. 865.

<sup>2</sup> Ibid., 1916, 30, p. 681.

<sup>3</sup> Jour. Hygiene, 1918, 17, p. 13 (Organism No. 2).

<sup>4</sup> Weinberg and Séguin, La Gangrène Gazeuse, 1917.

speak of a fermentation of glycerol as well, acid alone being produced. Gelatin and other enriched protein mediums are rather deeply decomposed.

Two cultures are studied in this series:

B. bifermentans A, from Miss Hemple.

B. bifermentans B, from Miss Hemple.

They were typical morphologically, and they induced a slow, but definite gaseous fermentation in glucose, maltose and glycerol. The volume of gas was smaller in glycerol than that observed in the sugar mediums. Gelatin was liquefied, and a soluble proteolytic enzyme was obtained from filtrates of the gelatin cultures which would induce liquefaction in a solution of gelatin in 0.5% phenol solution. In such a medium the bacteria of course could not grow. Consequently, there was no increase in ammonia. The amino-nitrogen content of the medium, however, increased somewhat, indicating a gradual breaking of the protein tie in the gelatin molecule with a coincident liberation of free  $\text{NH}_2$  groups as mono-amino acids, and as polypeptids of varying degrees of complexity. As the amino-acid content of gelatin cultures decreased during bacterial growth while it increased in the carbol gelatin containing the soluble enzyme of the organism but no live bacteria, it would appear that the amino acids and polypeptids formed as a result of the cleavage of the protein molecule were utilized as sources of energy by the microbes. It will be noticed in the analytic table that the amino nitrogen of cultures containing no utilizable carbohydrate shows a moderate but definite decrease as a result of bacterial development with a concomitant formation of ammonia. In mediums containing utilizable carbohydrate—glycerol and glucose—on the contrary, the amino nitrogen does not undergo a diminution, and the concomitant ammonia formation is proportionately lessened.

This phenomenon is another manifestation of the sparing action of utilizable carbohydrate for protein as a source of energy for bacteria. In milk, in spite of the fact that the lactose is not apparently utilizable for the microbes, there is an increase in titratable acidity and a moderate increase in amino nitrogen formation. It would seem probable that a part of this carbohydrate type of reaction is due to the small but definite amount of glucose in the milk. Available evidence suggests also that B. bifermentans is able to effect a cleavage of fats, and the subsequent fermentation of glycerol resulting therefrom is possibly a factor in the accumulation of acid.



TABLE 1  
BACILLUS FERMENTANS

Day	Plain			Gelatin			Glucose			Mannitol			Glycerol			Lactose			Saccharose			Starch			Milk		
	Reaction	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Amino Nitrogen	Percentage Ammonia to Total Nitrogen
Control.....	-0.60	10.5	27.3	3.95	-0.40	7.0	30.8	1.19	0.00	10.5	27.3	3.95	-0.60	10.5	27.3	3.95	-0.60	10.5	27.3	3.95	-0.60	10.5	27.3	3.95	-0.60	10.5	27.3
Culture A....	+0.40	39.2	20.3	14.7	+0.50	22.4	25.2	8.52	+2.00	14.0	31.5	5.23	+2.50	18.8	31.5	6.82	+0.40	31.5	23.8	11.8	+0.40	39.2	19.6	14.7	+0.80	51.1	14.0
1	+0.60	65.1	19.6	24.4	+0.80	36.4	24.5	5.70	+2.40	18.2	29.4	6.84	+3.10	18.2	32.9	6.84	+1.00	58.1	19.6	21.9	+1.10	65.1	18.9	17.6	+1.10	67.2	16.1
3	+1.10	65.5	19.6	25.0	+1.00	53.2	23.1	8.35	+2.50	18.9	31.5	7.10	+3.10	18.2	34.3	6.84	+1.20	65.9	18.9	21.7	+1.10	68.2	19.6	14.7	+1.10	71.1	16.1
6	+1.10	75.1	20.3	25.2	+1.40	73.3	23.9	11.9	+3.00	19.6	31.5	7.37	+3.10	18.2	31.5	6.84	+1.20	70.7	20.3	23.7	+1.10	66.5	21.9	25.0	+1.20	79.8	18.9
13	+0.90	71.4	21.7	26.8	+1.20	56.7	26.6	8.91	+2.80	21.7	32.9	8.16	+2.60	18.9	31.5	7.10	+1.00	68.0	19.6	23.7	+1.10	71.4	21.7	26.8	+1.00	71.9	19.6
20	-0.70	11.9	27.3	4.25	-0.80	7.7	28.7	1.14	-0.20	11.9	27.3	4.25	-0.70	11.9	27.3	4.25	-0.70	11.9	25.9	4.25	-0.70	11.9	27.3	4.25	-0.70	11.9	27.3
Control.....	-0.60	10.5	27.3	3.95	-0.40	7.0	30.8	1.19	0.00	10.5	27.3	3.95	-0.60	10.5	27.3	3.95	-0.60	10.5	27.3	3.95	-0.60	10.5	27.3	3.95	-0.60	10.5	27.3
Culture 2....	+0.20	35.7	19.6	12.7	-0.80	8.4	29.4	1.25	+1.80	11.9	30.8	4.25	+1.50	9.8	31.5	8.50	+0.40	38.7	18.9	10.2	+0.40	37.5	20.3	12.5	+0.50	31.5	20.3
1	+0.50	50.4	18.9	15.0	+0.10	36.4	25.9	5.42	+2.20	16.1	28.7	5.75	+2.10	14.7	28.4	5.23	+0.70	42.0	19.6	15.0	+0.70	49.7	21.0	17.7	+0.80	56.7	19.6
3	+0.90	58.1	19.6	20.7	+0.70	59.2	25.9	8.58	+2.70	16.8	29.4	6.00	+2.60	14.7	28.4	5.23	+0.80	52.5	21.0	18.7	+0.80	59.5	22.4	13.2	+1.40	53.7	17.5
6	+0.90	58.1	19.6	20.7	+0.80	95.2	24.5	11.4	+2.80	14.0	30.1	5.00	+2.60	14.7	28.4	5.23	+1.10	61.6	19.6	22.0	+1.30	65.1	22.4	12.5	+1.10	66.5	21.0
13	+0.90	65.1	20.3	23.2	+0.70	94.5	23.1	11.0	+2.70	18.9	29.4	6.75	+2.70	21.7	34.3	7.75	+1.10	64.4	21.7	23.0	+1.30	70.0	23.1	12.5	+1.10	70.7	21.7
20	+0.90	65.1	20.3	23.2	+0.70	94.5	23.1	11.0	+2.70	18.9	29.4	6.75	+2.70	21.7	34.3	7.75	+1.10	64.4	21.7	23.0	+1.30	70.0	23.1	12.5	+1.10	70.7	21.7

Reaction: alkaline to neutral red; c normal acid or alkali per 100 c.c. of medium.

Ammonia and amino nitrogen expressed in milligrams per 100 c.c. of culture medium.

Control indicates initial composition of medium. Net results may be obtained by subtracting the control from the day's analysis.

Amino nitrogen is corrected in each instance for ammonia.

The titratable acidity of all mediums increases, and some gas is evolved. The increase in titratable acidity in glucose and glycerol mediums, which are fermentable by *B. bifermentans*, are, however, marked and unmistakable.

## SUMMARY

*B. bifermentans* is an anaerobic bacillus exhibiting, as its name suggests, both carbohydrophilic and proteophilic characteristics.

The former are indicated by a gaseous fermentation of glucose and glycerol with the concomitant development of acid and the production of a mucinous substance which accumulates in these cultures.

The proteophilic properties are manifested by the formation of a soluble gelatin-liquefying enzyme which possesses the property of cleaving the gelatin molecule with the gradual liberation of  $\text{NH}_2$  groups, which are detectable and measurable by the method of formol titration.

## BACILLUS OEDEMATIENS

## STUDY LII

*Bacillus oedematis* was isolated from several cases of gas gangrene by Weinberg and Séguin<sup>1</sup> early in the war. It was soon discovered that the organism produces a soluble poison, formed during the first 2 days of growth, which will kill guinea-pigs within 48 hours, as a rule. This soluble poison, like that of the Welch bacillus and *Vibrio septique*, loses its potency on prolonged incubation. It would appear, therefore, that the poison is a substance formed incidental to the growth of the organism rather than a toxin produced as a result of the utilization of protein for energy by the organism.<sup>2</sup>

*B. oedematis* appears to be very similar to, or identical with Novy's *B. oedematis maligni* II.<sup>3</sup> Weinberg and Séguin<sup>4</sup> also suggest a possible identity of their organism with Costa and Troisier's "*Bacillus Neigeux*,"<sup>5</sup> and with Aschoff's "*Gas oedema bacillus*."<sup>6</sup> The organism has been isolated by a number of investigators from the bacterial flora of infected wounds, and as it has been recovered from the soil, it is

<sup>1</sup> Compt. rend. Soc. biol., 1915, 78, p. 274, p. 507.

<sup>2</sup> Kendall, Day and Walker: Study XLIV, Jour. Infect. Dis., 1922, 30, p. 141.

<sup>3</sup> Ztschr. f. Hyg. u. Infektionskr., 1894, 17, p. 209.

<sup>4</sup> La Gangrène Gazeuse, 1917.

<sup>5</sup> Compt. rend. Soc. biol., 1915, 78, p. 352.

<sup>6</sup> Veröffentl. a. d. Geb. d. Mil. Sanitätswesens, 1918, 68, p. 1.

not surprising to find *Bacillus oedematiens* among the microbes of infected gunshot wounds.

Morphologically, *B. oedematiens* resembles the Welch bacillus rather closely. On the whole, it is longer and the ends are usually more rounded, however. The shorter individuals observed in rapidly growing cultures, when segmentation is taking place rapidly, may, however, appear more nearly oblong. The ends of such organisms are more nearly square cut, and at this stage of development it would be difficult indeed to distinguish the microbe from the gas bacillus (*Bacillus welchii*). The spores of *B. oedematiens* occur at one end of a bacillary rod, or, more accurately, the residual bacillary substances project from one pole of the spore, which is oval and greater in diameter than the parent cell. The mature spore with its adherent parental cell resembles a tennis racquet quite closely. The Welch bacillus spore, although somewhat greater in diameter than the parent cell, is usually central or subterminal. The spore bacillary rod complex in this instance consists of a fragment of bacillary substance at each end of the oval, distended spore. *B. oedematiens* sporulates much more readily than *Bacillus welchii*. Weinberg and Séguin<sup>4</sup> state that the former will produce mature spores even in glucose-containing mediums. The cultures identified and discussed as *B. oedematiens* in this study failed to form spores in mediums containing utilizable sugars; at least, spores were not observed under these conditions, although search was made for them.

Culturally, *B. oedematiens* is not very distinctive. Weinberg and Séguin<sup>4</sup> state that gas and acid are produced in mediums containing glucose, levulose and maltose. Henry<sup>7</sup> states that starch and xylose are also fermented. The cultures described in the following failed to ferment starch. Xylose was not investigated.

Wolf<sup>8</sup> has studied the metabolism of *B. oedematiens* in peptone solution, glucose peptone solution, milk, and the cooked meat medium. His experiments show that the organism produces considerably less nitrogenous change in a simple peptone medium than in the cooked meat medium, containing much more highly complex protein. This is indicated both by the greater amount of ammonia formed, and by the accumulation of amino nitrogen. The addition of glucose to peptone solution clearly spares the protein constituents of the medium, shown both by a lessened ammonia formation and amino nitrogen content.

<sup>7</sup> Jour. Path. & Bacteriol., 1916, 21, p. 344.

<sup>8</sup> Ibid., 1919, 23, p. 254.

Day	Platin			Gelatin			Glucose			Mannitol			Glycerol			Lactose			Saccharose			Starch			Milk		
	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen
Control.....	-0.60	3.5 23.1	1.78	-0.50	3.5 39.9	0.89	-0.20	3.5 18.9	1.78	-0.60	3.5 21.7	1.78	-0.40	2.8 22.4	1.42	-0.50	3.5 21.7	1.78	-0.60	3.5 21.7	1.78	-0.60	3.5 21.7	1.78	-0.60	3.5 21.7	1.78
Culture A.....	+0.20	0.3 18.9	3.21	+0.50	12.6 43.4	1.41	+1.40	5.6 22.4	2.85	+0.30	5.6 18.2	2.85	+1.00	10.5 24.5	5.34	+0.20	8.4 19.6	4.28	+0.30	5.6 17.5	2.85	+0.20	7.7 23.1	3.63	+1.70	14.0 24.5	3.03
1	+0.20	23.1 24.5	11.7	+0.80	37.8 56.7	4.60	+1.40	9.1 23.1	4.64	+0.30	16.8 20.3	8.38	+1.00	14.7 25.2	7.56	+0.20	12.6 20.3	6.42	+0.30	16.8 20.3	8.38	+0.30	18.9 26.6	9.63	+2.30	29.3 25.0	6.33
3	+0.40	36.8 25.2	13.6	+1.20	44.5 63.0	5.20	+1.70	11.9 23.1	6.07	+0.30	35.2 21.7	12.8	+1.20	15.4 29.4	7.86	+0.40	31.3 21.0	16.0	+0.30	27.3 23.3	13.9	+0.30	31.5 26.6	16.0	+2.30	33.6 31.5	7.23
6	+0.50	42.0 25.2	21.4	+1.50	54.6 61.6	6.11	+1.40	12.6 21.7	6.42	+0.30	34.3 23.8	17.5	+1.20	16.8 28.7	8.57	+0.40	39.9 23.8	20.3	+0.30	35.0 21.7	17.8	+0.30	31.5 26.6	16.0	+1.90	42.0 42.0	9.09
Control.....	-0.50	3.5 23.1	2.08	-0.70	4.2 46.2	0.44	-0.20	3.5 21.7	2.08	-0.50	3.5 23.1	2.08	-0.50	3.5 23.1	2.08	-0.50	3.5 23.1	2.08	-0.50	3.5 23.1	2.08	-0.40	3.5 21.7	2.08	-0.40	3.5 21.7	2.08
Culture B.....	+0.10	8.4 16.1	5.00	+0.70	14.7 41.3	1.54	+1.10	8.4 25.2	5.00	+0.10	7.7 18.9	4.57	+0.10	7.7 16.8	4.57	+0.20	8.4 17.5	5.00	+0.10	5.6 17.5	6.22	+0.20	11.2 17.5	6.67	+1.80	9.8 21.0	0.88
1	+0.20	27.3 21.0	16.2	+1.00	30.8 63.0	3.23	+1.00	10.5 26.6	6.22	+0.20	23.5 23.8	14.1	+0.40	24.5 26.6	14.5	+0.20	23.1 18.2	13.7	+0.20	21.7 18.9	10.1	+0.30	28.0 19.6	16.6	+0.40	31.5 26.6	6.62
3	+0.40	81.5 22.4	16.7	+1.30	44.8 68.6	4.71	+1.50	12.6 25.2	7.50	+0.40	35.2 24.0	20.8	+0.60	27.3 26.6	16.2	+0.40	32.3 23.8	19.1	+0.40	32.3 23.8	19.1	+0.60	35.7 20.3	21.2	+2.50	36.4 28.7	7.65
6	+0.30	85.5 27.3	21.9	+1.50	56.0 65.8	5.88	+1.50	15.4 25.2	9.17	+0.30	37.8 28.7	22.5	+1.10	33.6 32.9	20.0	+0.30	36.4 23.8	21.6	+0.30	36.4 23.8	21.6	+0.40	38.5 24.5	21.9	+2.30	45.5 35.0	9.35
13	.....	.....	.....	+1.60	56.3 64.4	5.79	+1.30	16.8 23.8	10.0	+0.20	41.3 29.4	24.5	+1.00	36.4 35.0	21.6	+0.20	30.9 24.5	23.7	+0.30	37.8 25.2	22.5	+0.30	41.3 27.3	24.5	+2.00	48.3 39.2	10.1
21	.....	.....	.....	+1.70	59.3 62.3	6.59	+1.50	18.9 22.4	9.63	+0.20	35.2 21.7	1.78	-0.40	2.8 22.4	1.42	-0.50	3.5 21.7	1.78	-0.60	3.5 21.7	1.78	-0.60	3.5 21.7	1.78	-0.60	3.5 21.7	1.78
Control.....	-0.60	3.5 23.1	1.78	-0.50	3.5 39.9	0.89	-0.20	3.5 18.9	1.78	-0.60	3.5 21.7	1.78	-0.40	2.8 22.4	1.42	-0.50	3.5 21.7	1.78	-0.60	3.5 21.7	1.78	-0.60	3.5 21.7	1.78	-0.60	3.5 21.7	1.78
Culture C.....	+0.10	6.3 22.4	3.21	+0.30	16.8 35.0	0.89	+0.80	9.8 23.1	5.00	+0.10	9.1 18.9	4.64	+0.10	5.6 16.8	2.85	+0.10	16.8 16.1	8.58	+0.10	14.7 18.9	7.50	+0.10	16.1 16.8	8.21	+1.50	7.0 20.3	1.51
1	+0.20	28.7 21.0	14.6	+1.30	32.9 42.9	4.31	+1.20	11.2 24.5	5.77	+0.30	16.8 20.3	10.0	+0.60	5.6 22.4	2.85	+0.20	12.6 16.8	11.4	+0.20	14.7 18.9	11.0	+0.10	16.1 16.8	8.21	+2.60	37.8 23.0	8.18
3	+0.30	34.3 23.1	17.4	+1.60	47.6 63.7	5.35	+1.00	12.6 24.5	6.42	+0.30	16.8 20.3	13.5	+0.60	12.6 22.4	7.79	+0.20	12.6 16.8	11.4	+0.20	14.7 18.9	11.0	+0.10	16.1 16.8	8.21	+2.70	42.0 36.6	9.09
6	+0.30	45.5 23.8	23.2	+1.60	57.1 64.4	6.46	+1.70	17.5 25.0	8.83	+0.30	37.8 28.7	18.2	+0.60	12.6 22.4	7.79	+0.20	12.6 16.8	11.4	+0.20	14.7 18.9	11.0	+0.10	16.1 16.8	8.21	+2.60	43.5 32.2	9.85
13	+0.30	45.5 23.8	23.2	+1.60	57.1 64.4	6.46	+1.70	17.5 25.0	8.83	+0.30	37.8 28.7	18.2	+0.60	12.6 22.4	7.79	+0.20	12.6 16.8	11.4	+0.20	14.7 18.9	11.0	+0.10	16.1 16.8	8.21	+2.60	43.5 32.2	9.85
20	+0.30	46.2 25.2	23.5	+1.70	59.3 62.3	6.59	+1.50	18.9 22.4	9.63	+0.20	35.2 21.7	1.78	-0.40	2.8 22.4	1.42	-0.50	3.5 21.7	1.78	-0.60	3.5 21.7	1.78	-0.60	3.5 21.7	1.78	-0.60	3.5 21.7	1.78
Control.....	-0.50	3.5 23.1	1.85	-0.80	4.9 44.1	0.54	-0.20	3.5 21.7	1.85	-0.40	3.5 23.1	1.78	-0.40	2.8 22.4	1.42	-0.50	3.5 23.1	1.85	-0.40	3.5 23.1	1.85	-0.40	3.5 23.1	1.85	-0.40	3.5 23.1	1.85
Culture D.....	+0.10	14.7 22.4	7.82	+1.10	10.5 37.8	1.16	+1.20	10.5 22.4	5.56	+0.10	6.3 22.4	4.64	+0.80	12.6 24.5	6.67	+0.40	14.7 18.2	7.82	+0.40	14.7 21.0	7.82	+0.40	14.7 21.0	7.82	+1.60	14.7 21.7	2.80
1	+0.20	28.0 16.8	14.8	+1.40	41.3 56.0	4.57	+1.60	12.6 23.1	6.67	+0.20	18.2 19.6	10.0	+1.40	14.7 26.6	7.82	+0.30	14.7 18.2	7.82	+0.30	14.7 21.0	7.82	+0.40	14.7 21.0	7.82	+2.40	27.3 26.6	5.20
3	+0.30	34.3 25.9	15.1	+1.80	45.5 53.2	5.04	+1.40	14.0 27.3	7.41	+0.20	22.4 25.9	13.5	+1.00	17.2 28.9	9.14	+0.40	14.7 18.2	7.82	+0.40	14.7 21.0	7.82	+0.40	14.7 21.0	7.82	+2.60	35.7 28.0	6.80
6	+0.30	41.3 27.3	21.8	+1.80	53.8 67.9	6.51	+1.20	16.8 22.4	8.90	+0.20	30.8 21.7	18.2	+1.00	21.7 27.3	11.14	+0.20	14.7 18.2	7.82	+0.20	14.7 21.0	7.82	+0.40	14.7 21.0	7.82	+2.60	42.7 33.6	8.13
13	+0.30	43.4 26.6	22.9	+2.00	61.6 66.5	6.80	+1.20	16.1 22.4	8.52	+0.10	34.3 23.1	19.2	+1.20	21.0 29.4	11.11	+0.20	14.7 18.2	7.82	+0.20	14.7 21.0	7.82	+0.40	14.7 21.0	7.82	+2.60	45.5 41.3	8.67
21	+0.30	43.4 26.6	22.9	+2.00	61.6 66.5	6.80	+1.20	16.1 22.4	8.52	+0.10	34.3 23.1	19.2	+1.20	21.0 29.4	11.11	+0.20	14.7 18.2	7.82	+0.20	14.7 21.0	7.82	+0.40	14.7 21.0	7.82	+2.60	45.5 41.3	8.67
Control.....	-0.50	3.5 23.1	1.85	-0.80	4.9 44.1	0.54	-0.20	3.5 21.7	1.85	-0.40	3.5 23.1	1.78	-0.40	2.8 22.4	1.42	-0.50	3.5 23.1	1.85	-0.40	3.5 23.1	1.85	-0.40	3.5 23.1	1.85	-0.40	3.5 23.1	1.85
Culture E.....	+0.10	16.1 19.6	8.53	+0.80	10.5 43.4	1.16	+1.00	10.5 21.0	3.35	+0.40	8.4 23.1	4.44	+0.80	11.9 25.2	6.29	+0.50	14.7 18.2	7.82	+0.50	14.7 21.0	7.82	+0.40	16.8 17.5	8.89	+1.50	9.8 23.1	1.86
1	+0.20	32.8 18.9	20.7	+1.30	39.2 56.0	4.24	+1.60	7.7 23.1	4.07	+0.30	30.9 21.0	12.1	+1.00	14.7 28.7	7.77	+0.20	14.7 18.2	7.82	+0.20	14.7 21.0	7.82	+0.40	16.8 17.5	8.89	+1.50	9.8 23.1	1.86
3	+0.30	42.0 23.1	22.2	+1.70	45.5 59.5	5.04	+1.40	13.3 23.8	7.04	+0.40	41.3 28.0	21.8	+1.00	16.8 32.2	8.89	+0.20	14.7 18.2	7.82	+0.20	14.7 21.0	7.82	+0.40	16.8 17.5	8.89	+1.50	9.8 23.1	1.86
6	+0.30	42.0 23.1	22.2	+1.70	45.5 59.5	5.04	+1.40	13.3 23.8	7.04	+0.40	41.3 28.0	21.8	+1.00	16.8 32.2	8.89	+0.20	14.7 18.2	7.82	+0.20	14.7 21.0	7.82	+0.40	16.8 17.5	8.89	+1.50	9.8 23.1	1.86
13	+0.30	43.4 23.4	22.9	+1.90	60.4 64.4	6.47	+1.40	18.9 22.4	10.0	+0.40	46.8 25.9	24.7	+1.20	24.8 27.1	12.2	+0.50	14.7 18.2	7.82	+0.50	14.7 21.0	7.82	+0.40	16.8 17.5	8.89	+1.50	9.8 23.1	1.86
21	+0.30	44.1 23.1	23.3	+1.90	66.5 66.5	7.36	+1.00	20.3 21.0	10.7	+0.40	47.5 27.3	25.1	+1.10	23.1 27.3	12.2	+0.50	14.7 18.2	7.82	+0.50	14.7 21.0	7.82	+0.40	16.8 17.5	8.89	+1.50	9.8 23.1	1.86
Control.....	-0.50	3.5 23.1	1.85	-0.80	4.9 44.1	0.54	-0.20	3.5 21.7	1.85	-0.40	3.5 23.1	1.85	-0.40	2.8 22.4	1.42	-0.50	3.5 23.1	1.85	-0.40	3.5 23.1	1.85	-0.40	3.5 23.1	1.85	-0.40	3.5 23.1	1.85
Culture F.....	+0.00	15.4 19.6	8.15	+0.70	14.7 34.3	1.63	+1.00	5.6 25.2	2.96	+0.10	19.6 19.6	10.3	+1.00	11.9 26.6	6.39	+0.30	22.4 16.8	11.8	+0.30	22.4 16.8	11.8	+0.10	15.4 18.2	8.15	+1.40	14.0 21.7	2.66
1	+0.20	32.8 25.9	12.5	+1.40	39.2 54.6	3.28	+1.60	7.7 23.1	4.07	+0.20	25.9 23.1	13.7	+1.00	10.5 24.5	5.56	+0.10	12.6 19.6	11.8	+0.10	12.6 19.6	11.8	+0.10	15.4 18.2	8.15	+1.40	14.0 21.7	2.66
3	+0.30	36.8 25.2	13.6	+1.60	44.8 68.6	4.71	+1.50	12.6 25.2	7.50	+0.40	34.3 23.8	17.5	+1.20	16.8 28.7	8.57	+0.40	31.3 21.0	16.0	+0.40	31.3 21.0	16.0	+0.10	15.4 18.2	8.15	+1.40	14.0 21.7	2.66
6	+0.40	45.5 28.0	24.0	+2.00	61.6 66.5	6.80	+1.20	16.1 22.4	8.52	+0.10	34.3 23.1	19.2	+1.20	21.0 29.4	11.11	+0.20	14.7 18.2	7.82	+0.20	14.7 21.0	7.82	+0.40	16.8 17.5	8.89	+1.40	14.0 21.7	2.66
13	+0.40	45.5 28.0	24.0	+2.00	61.6 66.5	6.80	+1.20	16.1 22.4	8.52	+0.10	34.3 23.1	19.2	+1.20	21.0 29.4	11.11	+0.20	14.7 18.2	7.82	+0.20	14.7 21.0	7.82	+0.40	16.8 17.5	8.89	+1.40	14.0 21.7	2.66
21	+0.40	46.9 29.4	24.8	+2.00	63.0 66.0	6.47	+1.20	16.1 22.4	8.52	+0.1																	

Milk was fermented, according to Wolf's observations. His conclusions are that *B. oedematiens* exhibits both saccharolytic and proteolytic characteristics, the former greatly predominating. He notes, however, that "large quantities of gas are produced in cooked meat mediums containing no free carbohydrates. Notable quantities of amino acids and ammonia may be formed."

#### ORIGIN OF CULTURES

- B. oedematiens* A—From intestinal contents.
- B. oedematiens* B—From National Research Council.
- B. oedematiens* C—Culture incorrectly named *Bacillus chauvoei*.
- B. oedematiens* D—Culture incorrectly named *Bacillus welchii*.
- B. oedematiens* E—Culture incorrectly named *Bacillus* of Malignant oedema.
- B. oedematiens* F—Culture from National Research Council.

#### DISCUSSION

Milk is slowly changed by the growth of *B. oedematiens*. The reaction becomes acid, more rapidly with some strains, distinctly less rapidly with others. There is a tendency for the acid reaction to recede during the later days of incubation, although the reaction never reaches the initial titer. The casein is usually precipitated slowly, but the evidence of digestion of the clot is usually wanting. The reaction in reaction (see analytic table) appears to be associated with a gradual increase in the amount of ammonia formed as a result of the intracellular utilization of the protein constituents of the milk. There is some evidence of a parallelism between the development of acid in milk and in glycerol mediums. In both instances, the reaction becomes somewhat greater than in corresponding mediums not containing utilizable carbohydrate. Also, the amount of amino nitrogen detectable in cultures of the organism in milk and glycerol is greater than that found in other mediums, except gelatin. The suggestion is made that glycerol is slowly utilized as a source of energy with the liberation of acid substances which in turn give rise to the somewhat greater titratable acidity of these mediums. There appears to be justification for it in light of the quantitative differences in amino nitrogen and titratable acidity observed in these cultures. They do not seem to be accidental. This explanation is not without objections, however, and it is to be regarded as purely tentative.

The ammonia, except in glucose broth, is produced in moderate amounts which increase from day to day. The total accumulation is not very large, even on prolonged incubation, however. It is distinctly greater in mediums rich in complex nitrogenous substances, such as gelatin or milk, lesser in mediums containing protein derivatives of the order of peptones and polypeptids. It is perhaps to be expected that the evidences of intense, intracellular decomposition of protein would be lacking in cultures of *B. oedematiens* because the organism forms a soluble poison early in the growth of the culture. Intense proteolytic powers and toxin production appear to be incompatible.

In glucose broth, the amount of ammonia formed is distinctly less than in other corresponding mediums which do not contain a utilizable, non-nitrogenous source of energy. This is indicative of the sparing action of utilizable carbohydrate for protein.

The amino nitrogen detectable in the various mediums is moderate in amount, particularly with respect to those nutritive solutions whose nitrogenous constituents are peptones and polypeptids. A greater amino acid content is found in gelatin and milk cultures. The increase is suggestive of the greater suitability of complex protein for the energy requirements of the organisms. The greater amino-nitrogen content of glycerol and milk mediums has been discussed. In gelatin a considerable amount of amino nitrogen is characteristic of each of the six strains studied. The ammonia content of the medium is also greater than that of the others. Gelatin appears to be a suitable medium for the growth of *B. oedematiens*.

Acid is produced in all mediums irrespective of the nonprotein constituents; it is greater in peptone mediums containing glucose than in any other of the series studied. This is presumably due to the acid products arising from the utilization of the glucose for energy. The sparing action of the sugar for the protein is shown by the decrease in amino nitrogen in this medium in contrast to that of the mediums of the same nitrogenous composition but containing no utilizable non-nitrogenous source of energy. A similar but somewhat less intense action is observed in glycerol mediums, especially in cultures A, D, and F. Cultures B and C do not appear to be active fermenters of glycerol.

The chemical identification of *B. oedematiens* appears to be less precise than that of the purely fermentative or carbohdrophilic anaerobes. The organism exhibits moderate fermentative activities both with reference to its ability to utilize the commonly used sugars,

and with respect to the intensity of the reaction induced in these carbohydrates. Also, its proteophilic qualities are moderate. There is no evidence of a rapid deep-seated degradation of protein, although gelatin and the proteins of milk are progressively decomposed with the formation of gradually increasing amounts of ammonia and a corresponding increase of amino nitrogen.

The recognition of *B. oedematiens* would appear to rest largely on its morphology and ability to produce a soluble poison. For confirmation, the rather negative chemistry of the organism in contrast to *B. welchii* and *Vibrio septique*, would be a feature of importance. The differentiation from *Bacillus botulinus* would hinge directly on the labile nature of the poison of *Bacillus oedematiens* in contrast to the cumulative development of soluble toxin in cultures of *B. botulinus*, as well as the resistance of the latter to gastro-intestinal digestion.

## BACILLUS AEROFOETIDUS

### STUDY LIII

*Bacillus aerofoetidus* was first described by Weinberg and Séguin<sup>1</sup> as *Bacillus D*. Later they conferred the name "aerofoetidus" on it. Henry<sup>2</sup> confirmed the occurrence of *B. aerofoetidus* in infected wounds of warfare, and redetermined and extended the fermentation reactions of the organism, previously made by McIntosh and Feldes,<sup>3</sup> that glucose, maltose, and lactose were fermented with the production of considerable gas and acid by adding levulose and salicin to the list of non-nitrogenous sources of energy.

Weinberg and Séguin failed to detect spores in cultures of *aerofoetidus*, but Henry, and McIntosh and Feldes, found occasional spores which were subterminal, oval and apparently slightly greater in diameter than the parent bacterial cell. The cultures identified as *B. aerofoetidus* in the series reported in the following sporulated sparsely in mediums not containing utilizable carbohydrates.

The organism resembles *B. fallax* in its morphology, but it differs culturally in several particulars. The foul odor of protein cultures of *B. aerofoetidus* seems to have been one of the prominent qualitative features characteristic of the microbe, and it is to this factor that Weinberg and Séguin were led to formulate the specific name of the organism.

<sup>1</sup> Compt. rend. Soc. biol., 1916, 79, p. 116; La Gangrène Gazeuse, 1917.

<sup>2</sup> Henry: Jour. Path. & Bacteriol., 1916, 21, p. 367.

<sup>3</sup> Med. Res. Committee, Special Report Series 19, 1917.



TABLE 1  
BACILLUS AEROBETIDUS

Day	Plain			Gelatin			Glucose			Mannitol			Glycerol			Lactose			Saccharose			Starch			Milk			
	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen	
Control.....	-0.70	13.3	25.9	5.59	-0.60	9.8	39.2	1.21	-0.40	12.6	25.2	5.30	-0.70	11.9	25.9	5.00	-0.60	11.2	25.2	4.71	-0.70	11.9	25.9	5.00	-0.70	11.9	25.9	5.00
Culture A....	+0.20	22.4	15.6	9.43	+0.20	27.3	25.2	3.39	+1.80	14.0	27.3	5.89	+0.10	11.9	25.9	5.00	+0.40	21.0	16.8	8.83	+0.40	27.3	14.7	11.4	+0.40	21.0	16.8	8.83
1	+0.40	62.3	22.4	26.1	+1.50	97.3	31.5	12.1	+2.50	17.5	35.7	7.35	+0.40	45.4	13.3	18.2	+0.70	18.2	23.8	7.65	+0.30	59.5	16.3	24.9	+0.70	53.9	18.9	22.6
3	+0.40	61.6	23.8	25.9	+1.80	145.6	32.9	18.1	+2.70	18.2	37.9	7.65	+0.70	78.6	23.8	33.0	+0.60	24.5	27.3	10.3	+0.50	65.1	19.6	27.3	+0.80	79.1	22.4	33.2
6	+0.60	77.7	26.6	32.6	+2.00	219.6	39.9	27.3	+3.10	19.6	37.2	8.23	+0.80	80.5	26.8	33.8	+0.40	35.0	26.6	14.7	+0.60	85.4	21.7	35.8	+0.90	81.9	24.5	34.4
13	+0.70	79.8	28.7	33.5	+1.90	224.7	44.8	27.9	+3.30	19.6	39.5	8.23	+0.90	79.9	28.0	33.5	+0.50	42.7	24.5	17.9	+0.50	85.4	23.6	35.8	+0.70	84.0	27.5	35.2
20	-0.20	13.3	25.9	5.59	-0.60	9.8	39.2	1.21	-0.80	12.6	25.2	5.30	-0.70	11.9	25.9	5.00	-0.60	11.2	25.2	4.71	-0.70	11.9	25.9	5.00	-0.70	11.9	25.9	5.00
Control.....	+0.30	21.7	15.4	9.12	+0.10	22.4	31.5	2.78	+2.10	13.3	27.3	5.59	-0.70	8.4	23.8	8.52	+0.10	21.0	17.5	8.83	+0.70	18.9	20.3	7.94	+0.50	23.1	14.7	9.72
Culture B....	-0.70	53.2	18.2	22.3	+1.60	78.4	36.4	9.75	+2.40	14.7	28.0	6.18	-0.20	37.8	16.1	15.8	+0.80	37.8	25.9	15.8	+0.70	19.6	25.9	8.23	+0.50	46.2	16.3	19.4
1	-0.60	50.4	19.6	21.1	+2.00	117.6	42.7	14.6	+2.70	19.6	31.5	8.23	+0.30	51.1	19.6	21.1	+1.40	50.4	23.1	21.1	+0.90	31.5	27.3	13.2	+0.40	46.0	20.3	20.5
3	-0.60	63.0	25.2	26.4	+2.20	218.4	44.8	27.1	+2.90	19.6	36.4	8.23	+0.80	72.8	24.5	30.5	+1.40	60.2	23.8	25.2	+0.90	42.0	26.0	17.6	+0.70	72.8	22.4	30.5
7	+0.50	65.1	27.8	27.3	+2.10	226.1	47.6	28.1	+3.10	21.7	36.4	9.12	+0.80	77.7	26.6	32.6	+1.80	69.3	30.8	29.1	+0.70	42.0	26.6	17.6	+0.50	70.6	27.3	29.4
13	-0.70	53.2	18.2	22.3	+1.60	78.4	36.4	9.75	+2.40	14.7	28.0	6.18	-0.20	37.8	16.1	15.8	+0.80	37.8	25.9	15.8	+0.70	19.6	25.9	8.23	+0.50	46.2	16.3	19.4
20	-0.50	65.1	27.8	27.3	+2.10	226.1	47.6	28.1	+3.10	21.7	36.4	9.12	+0.80	77.7	26.6	32.6	+1.80	69.3	30.8	29.1	+0.70	42.0	26.6	17.6	+0.50	70.6	27.3	29.4

Reaction: — = alkaline to neutral red; + = acid to neutral red; c c normal acid or alkali per 100 c c of medium.

Ammonia and amino nitrogen expressed in milligrams per 100 c c of culture medium.

Control indicates initial composition of medium. Net results may be obtained by subtracting the control from the day's analysis.

Amino nitrogen is corrected in each instance for ammonia.

Culturally, *B. aerofœtidus* is of the saccharo-proteolytic type—it ferments some of the more commonly used carbohydrates with the production of considerable acid and gas, and it is a moderately proteoclastic organism as well, producing a soluble proteolytic enzyme which will effect the liquefaction of gelatin in the absence of the microbes.

*Origin of Cultures.*—Culture *B. aerofœtidus* "A" was obtained from Dr. Holman. It came originally from Paris.

Culture *B. aerofœtidus* "B" was obtained from the National Research Council. It is said to have been isolated from an infected wound.

#### DISCUSSION

The nitrogenous changes induced by *B. aerofœtidus* in mediums containing peptoné and meat extractives as the source of nitrogen are characterized by a considerable liberation of ammonia. The addition of utilizable carbohydrate (glucose) effects a decided reduction in the amount of this substance formed, suggesting a sparing action of the non-nitrogenous source of energy for the protein. At the same time the fermentative activities of *B. aerofœtidus* do not appear to be as marked in mediums containing utilizable protein as those corresponding for *B. welchii* and the *Vibrio septique*, which are carbohydrophilic rather than proteolytic in their activities. It appears to be a rather general characteristic of bacteria which produce soluble proteolytic enzymes to effect a deeper cleavage of the protein molecule even in the presence of utilizable carbohydrate than occurs with the less actively proteolytic types.

*B. proteus*, for example, forms relatively more ammonia (greater deamination) when it utilizes protein for energy than does *B. coli* under similar circumstances, and *B. coli* in turn forms more ammonia under like conditions than *B. diphtheriae*. It will be remembered that *B. proteus* is more proteophilic than *B. coli*; and *B. coli* in turn effects a deeper degradation of protein than *B. diphtheriae*. Vigorous decomposition of protein is incompatible, apparently, with toxicogenesis. *B. aerofœtidus* partakes of the proteophilic type. In gelatin the amount of ammonia formed is relatively large, amounting to a material proportion of the total nitrogen of the medium. Culture A is rather more active in this respect than culture B.\*

\* Slight variations in the intensity of proteolysis even in the same kind of microbe are common. Cultures of *B. proteus* are noteworthy in this regard. The older division of *proteus* bacilli into *B. proteus-vulgaris*, *micrabilis*, *zenkeri* and *zopfii* in a descending scale of proteolytic activity was based on the qualitative proteolytic powers of current strains of the *proteus* bacillus. It is now surmised that the four "species" are naturally occurring variants of the same organism, which tends to lose its exuberant proteolytic powers on prolonged cultivation in cultural mediums.

Cultures of *B. aerofœtidus* exhibit a decided reduction in the amount of amino nitrogen in mediums containing no utilizable carbohydrate during the first days of growth. Even in gelatin, in which ammonia formation (deamination) proceeds with relatively great rapidity, this phenomenon is clearly discernible. In mediums containing utilizable carbohydrate, on the contrary, such as glucose and lactose broths and in milk (which of course contains lactose) the amino nitrogen increases during incubation. That this phenomenon is associated with the sparing action of carbohydrate for protein would appear to be almost certain.

The reaction (titratable acidity) becomes progressively acid even in nonsaccharine mediums, but in mediums containing utilizable carbohydrates the acidity increases more rapidly and reaches a greater concentration than is the case in corresponding mediums containing no utilizable sugars.

*B. aerofœtidus*, to summarize, appears to be an organism whose primary action is proteolytic. Certain carbohydrates—such as glucose and lactose—can be utilized by it for energy, thereby reducing noticeably the attack on protein. The organism would appear to be best classified as being of the proteophilic anaerobic bacilli.

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## BACILLUS SPOROGENES

### STUDY LIV

*Bacillus sporogenes*, or that group of organisms which resembles this type of proteophilic microbe very closely, was first isolated from the intestinal contents and described by Metchnikoff.<sup>1</sup> In reality, Metchnikoff described two organisms, differing somewhat in morphology and in colony formation. One of these, which he called type A, was pictured as a rather slender rod, sometimes occurring in short chains, with subterminal spores. The other, called type B, was said to be stouter with centrally placed spores. The bacilli were studied chemically by Berthelot.<sup>2</sup> It was stated that both type A and B fermented glucose, galactose, maltose, lactose, and the alcohol mannitol; saccharose and starch were unfermented. McIntosh and Feldes<sup>3</sup> also described two anaerobic bacilli, one of which (No. XI) was identified

<sup>1</sup> *Ann. Inst. Past.*, 1908, 22, p. 929.

<sup>2</sup> *Ibid.*, 1909, 23, p. 85.

<sup>3</sup> *Med. Res. Committee, Special Report 12*, 1917.

by them as *B. sporogenes*. A second, unidentified bacillus with central spores (No. XII) exhibited quantitative rather than qualitative differences from their No. XI. They state, however, that the two are serologically unlike. Donaldson's "Reading bacillus"<sup>4</sup> (used by him to remove necrotic tissue from infected wounds, due to its soluble proteoclastic enzyme) is also in all probability a member of the *B. sporogenes* group.<sup>5</sup> The great problem confronting investigators of anaerobic bacteria is not that of obtaining cultures of *B. sporogenes*, however; it is to keep this organism out of cultural mediums and stock cultures of other anaerobes. The microbe is widely disseminated in nature, it forms resistant spores, and the most disagreeable and characteristic feature of all is its ability to grow in association with almost all other anaerobes as an unrecognized contaminant. Such "pure mixed cultures" containing unrecognized *B. sporogenes* in strains of *B. welchii*, *Vibrio septique*, *B. chauvoei*, *B. tetani*,<sup>6</sup> that organism called "Bacillus putrificus," *B. botulinus* and *B. fallax*, are fruitful sources of controversy. Of these cultures of the fermentative type of anaerobic bacilli, those first named are more commonly infected with *B. sporogenes* as an impurity. From the standpoint of the metabolism of anaerobic bacilli, it may be stated dogmatically that any culture containing living bacteria which shows little proteolysis for a week or 10 days followed by a sudden and noteworthy jump in the chemical evidences of proteolysis is contaminated with *B. sporogenes*, or somewhat less commonly, another of the proteophilic anaerobic bacilli.

In addition to the chemical observations of Berthelot,<sup>2</sup> Wolf and Harris<sup>5, 7</sup> have studied the metabolism of a strain of *B. sporogenes*, obtained from Henry.<sup>8</sup> Observations were made in various mediums, including milk and peptone broth. In milk they found a moderate and deliberate evolution of gas, gradually increasing in volume for 5 days. At the end of that period the amount of gas evolved was equal in amount to a third, or even a half, the original volume of milk. The gas was mainly CO<sub>2</sub> and H<sub>2</sub> in the proportion of about  $\frac{\text{CO}_2}{\text{H}_2} = 2/1$ . A striking feature of the reaction was a progressive increase in titratable acidity, as the incubation was protracted. The previous statements of reaction in milk were almost unanimously indicative of an alkaline

<sup>4</sup> Jour. Path. & Bacteriol., 1918, 22, p. 129.

<sup>5</sup> Harris: Jour. Path. & Bacteriol., 1919, 23, p. 30.

<sup>6</sup> Donaldson (footnote 4) states that *B. sporogenes* destroys, or at least reduces, the potency of tetanus toxin as a result of its growth in mediums containing the toxin.

<sup>7</sup> Jour. Path. & Bacteriol., 1916, 21, p. 386.

<sup>8</sup> Ibid., p. 359.

change. The cultures studied in the following series are in accord with this increase in titratable acidity.

Wolf and Harris found also that the ammonia production was considerable; in this respect, *B. sporogenes* stands in marked contrast to the fermentative type of organisms, as for example, *B. welchii*.

The amino nitrogen also was found to be greatly increased; it amounted, according to the figures presented, to more than twice that of the ammonia increase. In plain peptone mediums, on the contrary, a distinct loss of amino nitrogen occurred. Wolf and Harris,<sup>5, 7</sup> in discussing the formation of gas in milk, make the statement that "*Bacillus sporogenes* possesses a ferment which hydrolyzes this sugar (lactose) to glucose and galactose." As the fermentation of glucose and lactose solutions (glucose and lactose bouillon) were apparently not tried out in the experiments recorded, the evidence for this statement is based exclusively on a slight increase in reducing power of the milk cultures after incubation, as shown by the Fehling test. Approximately the same volume of gas was obtained by them from alkaline casein solution; therefore, it would not seem necessary to bring in a cleavage of lactose to explain the evolution of gas in milk or protein solutions. As a matter of fact, *B. sporogenes* seems to possess the ability to generate gas in any protein solution, provided a sufficient amount of the cultural medium is inoculated to detect the gas. Amounts less than 100 c c are unsuited for this purpose.

The salient features of Wolf and Harris' study of *B. sporogenes* indicate that the organism is an energetic deaminizer of protein (or protein derivatives) and that in mediums relatively rich in protein, such as milk or alkaline casein, a considerable increase in amino nitrogen takes place. In mediums containing less highly organized nitrogen, such as peptone solution, an actual deficit in amino nitrogen occurs. Berthelot<sup>2</sup> called attention to the deep-seated changes *B. sporogenes* induces in protein, and Donaldson<sup>4</sup> demonstrated the presence of a soluble proteolytic enzyme. Wolf and Harris state: Ammonia was formed in such large quantity that it more than balanced the original amino-acids present in the medium. There is therefore a very vigorous deamination taking place, with an effort to keep the concentration of amino-acids up to the original level. Only one other explanation would be possible, namely, that bacteria attacked higher complexes and degraded them directly to ammonia, a type of reaction for which we have no analogy in higher metabolism." It appears reasonable to explain the relationship between ammonia formation and amino-nitro-

TABLE 1  
BACILLUS SPOROGENES

Day	Plain				Gelatin				Glucose				Mannitol				Glycerol				Lactose				Saccharose				Starch				Milk			
	Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen		Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen		Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen		Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen		Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen		Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen		Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen		Reaction	Ammonia Nitrogen	Percentage Ammonia to Total Nitrogen					
Control A	-0.60	22.4	29.4	6.25	-0.40	22.4	32.2	3.09	-0.20	22.4	28.0	6.25	-0.50	22.4	29.4	6.25	-0.50	22.4	29.4	6.25	-0.50	22.4	29.4	6.25	-0.50	22.4	29.4	6.25	-0.50	22.4	29.4	6.25	-0.50	22.4	29.4	6.25
	+1.00	36.0	20.3	15.6	+1.70	47.6	26.6	7.82	+3.50	37.2	23.0	5.10	+1.10	56.0	19.6	15.6	+1.10	49.0	23.1	13.7	+1.00	57.4	21.0	16.0	+1.00	57.4	21.0	16.0	+1.00	57.4	21.0	16.0	+1.00	57.4	21.0	16.0
	+2.00	110.6	21.0	30.9	+2.60	119.0	28.6	19.5	+4.50	37.1	21.7	7.04	+1.70	111.1	32.0	33.1	+1.90	101.7	23.1	28.4	+1.90	106.7	21.0	27.6	+2.00	105.7	18.9	29.6	+2.00	105.7	18.9	29.6	+2.00	105.7	18.9	29.6
	+3.00	128.8	25.5	28.6	+3.00	151.9	25.7	24.9	+3.50	56.0	35.0	15.6	+2.00	122.5	34.5	34.3	+3.90	32.2	35.0	9.9	+1.90	123.9	21.1	34.7	+2.30	118.7	23.1	35.8	+2.30	118.7	23.1	35.8	+2.30	118.7	23.1	35.8
	+4.00	147.0	33.6	41.1	+3.70	174.3	45.5	28.1	+3.50	0.7	35.7	19.7	+2.30	142.1	42.5	39.8	+2.70	35.0	34.3	9.8	+2.20	135.1	25.2	37.8	+2.70	128.1	24.5	40.8	+2.70	128.1	24.5	40.8	+2.70	128.1	24.5	40.8
20	+2.20	147.7	38.5	41.3	+3.20	166.0	43.4	32.1	+3.40	7.8	36.4	21.9	+2.30	142.1	31.5	39.8	+2.40	50.4	32.2	14.1	+1.80	135.5	30.8	38.0	+2.80	197.5	29.4	53.9	+2.00	145.6	30.8	40.8	+2.00	145.6	30.8	40.8
Control B	-0.90	29.4	35.0	10.5	-0.60	16.1	35.7	2.42	-0.50	29.4	32.2	10.5	+1.70	55.3	33.6	11.0	-0.90	9.4	35.0	10.5	-0.90	28.0	33.6	10.0	-0.90	30.1	28.0	10.7	-0.90	29.4	35.0	10.5	+1.50	6.3	18.6	1.36
	+1.70	60.9	38.5	52.17	+2.50	78.4	29.4	12.3	+3.10	32.2	30.8	11.5	+1.70	55.3	33.6	29.7	+2.90	27.3	31.5	9.75	+1.80	71.4	25.9	25.5	+2.10	71.4	25.9	25.5	+2.10	75.6	26.6	27.0	+1.60	9.1	18.9	1.96
	+2.10	95.9	29.7	34.2	+3.80	149.1	30.8	23.4	+3.70	35.0	30.1	11.7	+2.30	91.1	36.4	32.5	+3.60	30.8	32.2	11.0	+2.10	91.0	32.2	32.5	+2.40	105.7	29.7	31.5	+2.10	105.7	29.7	31.5	+2.10	105.7	29.7	31.5
	+2.50	108.9	27.7	39.2	+4.00	182.0	33.6	28.5	+3.60	54.6	37.5	19.5	+2.30	106.4	37.8	38.0	+3.80	40.8	35.7	14.5	+2.40	107.8	35.5	38.5	+2.70	114.8	41.3	41.0	+2.40	107.8	35.5	38.5	+2.40	107.8	35.5	38.5
	+2.90	126.9	31.9	43.0	+4.40	204.0	38.8	31.1	+3.90	72.8	49.7	26.0	+2.30	121.4	43.2	43.2	+3.10	41.3	37.8	14.7	+2.10	113.4	35.7	40.5	+2.70	112.0	40.6	40.0	+2.40	122.5	42.7	43.7	+2.10	122.5	42.7	43.7
20	+2.20	117.6	33.6	42.0	+3.90	309.4	42.7	48.6	+3.60	73.5	53.2	26.2	+2.30	119.0	34.3	42.5	+3.80	39.3	38.5	14.0	+2.20	119.7	38.5	42.7	+2.70	119.7	39.2	42.7	+2.10	133.0	43.4	47.5	+2.10	133.0	43.4	47.5

Reaction: — = alkaline to neutral red; + = acid to neutral red; cc normal acid or alkali per 100 cc of medium.

Ammonia and amino nitrogen expressed in milligrams per 100 cc of culture medium. Control indicates initial composition of medium. Net results may be obtained by subtracting the control from the day's analysis. Amino nitrogen is corrected in each instance for ammonia.

gen content of the mediums, on the probable effect of the proteoclastic enzyme of *B. sporogenes*. This cleaves the protein molecule to amino-acids, or polypeptids, thus continually renewing, as it were, the supply of amino-acids utilizable for energy. This supply of amino-acids is continuously deaminized (but at an unknown rate), and the results would be those obtained by Wolf and Harris in mediums rich in protein. In protein-poor mediums, such as peptone water, the supply of cleavable protein is much less, and consequently the deaminizing power is presumably greater in proportion, and an actual deficit in amino-acids below that of the uninoculated controls results.

*Origin of Cultures.*—Two cultures were studied in this series, A and B. Culture A was isolated from feces. Culture B was a contaminant obtained from an impure strain of the organism of symptomatic anthrax (*B. chauvœi*). Both cultures fermented glucose and maltose with the production of gas. Lactose, saccharose, starch and mannitol were not fermented with the formation of gas above that of nonsaccharine mediums.

It should be stated that a slow, gradual evolution of gas was observed in plain peptone bouillon, a somewhat greater liberation in mediums rich in protein, such as gelatin and milk. The reaction in glycerol became strongly acid, and although the gaseous evolution was not materially greater than that observed in plain broth, nevertheless the impression gained was that the organisms utilized glycerol as a non-nitrogenous source of energy. Organisms of the general type of *B. sporogenes* are difficult to classify with respect to their fermentation reactions, because the liberation of gas and development of progressive titratable acidity occur with moderate intensity in nonfermentation mediums. The excess of gas and acid in true fermentation mediums is measurable only by rigid comparison.

Both cultures contained a soluble, proteolytic enzyme which effected a fairly rapid cleavage of gelatin (carbol gelatin) in the absence of bacteria. This soluble enzyme is probably the most distinctive chemical characteristic of the organism. It appears early in the incubation of the medium, and it acts with considerable rapidity.

#### DISCUSSION

The analytic statistics of the metabolism of the two cultures of *B. sporogenes* exhibit a feature of proteophilic bacteria which deserves passing comment. Culture A is distinctly more active in all its mani-



festations than Culture B, although the differences are quantitative rather than qualitative.<sup>9</sup>

In milk, the nitrogenous changes are on the whole quite similar in type to those observed by Wolf and Harris—a marked deamination, indicated by the steady increase in ammonia and the decided increase in amino nitrogen. The former—ammonia formation—indicates the intracellular utilization of nitrogenous protein derivatives by the microbes, and the latter suggests strongly the action of the soluble proteolytic enzyme, mentioned previously. The action of this enzyme affords a relatively simple explanation for the simultaneous development of the bacteria, the rapid formation of ammonia, and the ascending tendency of the amino-acid content of the medium in spite of the probable utilization of amino acids or amino acid complexes by the bacteria for their combined structural and energy requirements. The reaction, in terms of titratable acidity, becomes progressively acid. This is true qualitatively in all mediums, however. The reactions in gelatin are much the same.

In mediums containing peptone and meat extractives as sources of nitrogen the reactions were less intense. The amino acid residuum was smaller from day to day in mediums not containing glucose or glycerol than those in the corresponding uninoculated controls. In other words, the organisms reduced the content of amino acids in mediums not containing highly organized nitrogen, such as gelatin or milk. It will be remembered that Wolf and Harris found the same phenomenon in peptone water cultures.

In mediums containing glucose or glycerol, on the contrary, the amino nitrogen content was nearly stationary, or even a little above the uninoculated controls. At the same time, the rate and amount of ammonia formation was distinctly less than that found in corresponding mediums containing no utilizable carbohydrate. This is suggestive of a sparing action of the carbohydrate for protein.

#### SUMMARY

*B. sporogenes* is a striking example of the group of the proteophilic anaerobic bacilli. It forms a potent, soluble proteolytic enzyme, which effects a relatively rapid cleavage of proteins, and it utilizes protein for energy with the liberation of considerable amounts of ammonia. In

<sup>9</sup> Harris (footnote 5) has also found this same phenomenon in his careful studies of the "*Reading bacillus*" and *B. sporogenes*.

mediums rich in protein—gelatin and milk—the amino acid content increases materially in spite of the utilization of the products of protein cleavage for both the structural and energy requirements of the organisms. In mediums containing protein of the peptone type, however, the amino-nitrogen content diminishes incidentally to the growth of the organisms; the ammonia formation, however, is of about the same rate and intensity in either type of medium. The addition of utilizable, non-nitrogenous sources of energy, such as glucose or glycerol, reduces noticeably both the formation of ammonia and the utilization of amino acids for energy.

## BACILLUS HISTOLYTICUS

### STUDY LV

*Bacillus histolyticus* is a member of the plectridial group of anaerobic bacilli. It was isolated and its most characteristic property described by Weinberg and Séguin<sup>1</sup> in 1915. *B. histolyticus* derives its name from the remarkable proteolytic ability it possesses of liquefying injured or necrotic tissue.<sup>2</sup> Legros and Vaucher<sup>3</sup> have observed this digestive action in the tissues surrounding wounds of warfare, and Nicolas<sup>4</sup> has made similar observations in horses. Unlike *B. sporogenes*,<sup>5</sup> however, which dissolves injured tissue without serious results to the patient, the presence of *B. histolyticus* in infected wounds leads frequently to serious signs and symptoms of toxemia, which may result fatally. The pathogenicity of strains, however, varies considerably. The more virulent strains cause a progressive necrosis which may eventually become widespread. Weinberg and Séguin<sup>6</sup> have found a soluble poison which appears in cultures of the organism during the earlier hours of incubation in its greatest potency. In this respect the soluble poison resembles that of *B. welchii* and *Vibrion septique*, rather than the soluble toxins of *B. tetani*, *B. botulinus*, and the diphtheria bacillus, which are cumulative and apparently formed as a result of the utilization of protein derivatives for energy.

The cultural identification of *B. histolyticus* appears to be a subject of controversy. Apparently the tissue-liquefying properties of the

<sup>1</sup> Compt. rend. Soc. biol., 1915, 78, p. 274.

<sup>2</sup> Ibid., 1917, 80, p. 157.

<sup>3</sup> La Gangrène Gazeuse, 1917, p. 318.

<sup>4</sup> Ibid., p. 319.

<sup>5</sup> Donaldson: Jour. Path. & Bacteriol., 1918, 22, p. 129. Donaldson and Joyce. Lancet, 1917, 2, p. 445.

<sup>6</sup> La Gangrène Gazeuse, p. 171.

TABLE 1  
BACILLUS HISTOLYTICUS

	Day	Plain				Gelatin				Glucose				Lactose				Saccharose				Milk			
		Reaction	Ammonia Nitrogen	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Amino Nitrogen	Percentage Ammonia to Total Nitrogen
Control	..	-0.70	11.9	27.3	4.72	-0.50	7.0	36.4	0.96	-0.40	11.9	25.9	4.72	-0.60	11.9	25.9	4.72	-0.70	11.9	27.3	4.42	+1.40	5.6	19.6	0.89
Culture A	1	+0.20	54.9	35.0	21.8	+1.50	161.7	78.4	23.1	+0.20	49.7	28.0	19.7	+0.20	50.4	30.1	20.0	+0.20	53.9	33.6	21.3	+1.40	7.0	21.0	1.12
	8	+0.10	67.2	34.3	26.6	+1.70	250.6	57.0	34.4	+0.10	58.1	32.2	23.0	+0.10	61.6	32.9	24.4	+0.10	65.8	31.5	26.1	+2.70	51.1	55.3	8.20
	6	+0.20	67.2	35.0	26.6	+1.70	269.5	54.6	37.0	+0.10	61.6	32.9	24.4	+0.10	61.6	32.9	24.4	+0.20	67.9	33.6	26.9	+2.80	90.3	80.5	14.5
	13	+0.10	70.7	37.8	28.2	+1.50	270.9	70.7	37.2	-0.10	62.3	35.0	24.7	0.00	65.8	33.4	26.1	+0.10	70.0	35.7	27.7	+2.80	100.1	83.3	16.2
	20	+0.20	72.8	39.9	28.8	+1.20	276.5	80.5	38.0	-0.20	63.7	35.0	25.2	-0.30	66.5	35.0	26.4	+0.20	72.1	35.0	28.5	+2.90	100.1	86.1	16.2
Control	..	-1.00	18.9	30.1	6.75	-0.70	11.9	37.1	1.54	-0.70	18.9	25.9	6.75	-0.90	16.1	27.3	5.72	-1.60	18.9	27.3	6.72	+1.20	3.5	21.7	0.80
Culture B	1	+0.40	51.8	34.3	18.4	+0.30	175.7	51.8	22.8	+0.50	45.5	29.4	16.2	+0.70	49.0	27.3	17.5	+0.70	49.7	32.9	17.7	+1.50	6.8	21.7	1.45
	8	+0.70	55.3	39.2	19.7	+0.50	249.2	94.1	32.4	+0.60	51.1	32.2	18.2	+0.70	53.9	33.6	19.2	+0.60	56.0	37.1	20.0	+1.80	46.2	35.0	10.6
	6	+0.70	59.5	39.2	21.2	+0.40	290.5	88.3	37.7	+0.50	54.6	37.1	19.5	+0.70	56.0	35.7	20.0	+0.50	58.8	35.7	21.0	+2.10	90.3	46.9	20.8
	13	+0.30	64.4	41.3	23.0	+0.20	298.6	119.0	38.2	+0.30	56.7	38.5	20.2	+0.30	56.0	35.7	20.0	+0.20	60.2	39.9	21.5	+2.40	100.8	79.1	23.2
	20	+0.10	65.8	45.5	23.5	+0.20	301.7	126.7	39.2	+0.30	58.7	42.7	20.9	+0.10	56.7	40.6	20.2	-0.10	63.7	44.8	22.7	+2.70	100.1	84.0	23.0
Control	..	-0.80	10.5	23.1	4.55	-0.90	11.2	42.0	1.33	-0.70	10.5	23.1	4.55	-0.70	10.5	23.1	4.55	-0.70	10.5	23.1	4.55	+1.30	3.5	20.3	0.74
Culture C	1	+0.20	48.3	27.3	21.0	+0.10	185.5	86.1	22.2	+0.20	47.6	20.6	20.6	.....	.....	.....	.....	.....	.....	.....	.....	+1.90	8.4	19.6	1.79
	3	+0.40	56.0	32.9	24.2	+1.40	272.3	86.1	32.4	+0.10	53.2	23.1	23.0	+0.20	51.1	22.4	22.1	+0.30	53.9	.....	23.3	+2.00	33.6	44.8	7.16
	7	+0.30	60.9	30.8	26.3	+1.90	290.5	77.7	34.6	+0.60	56.0	24.5	24.2	.....	.....	.....	.....	.....	.....	.....	.....	+2.20	57.4	56.0	12.2
	10	+0.80	59.5	33.6	25.9	+1.80	350.3	81.9	35.7	+0.70	56.0	24.5	24.2	.....	.....	.....	.....	.....	.....	.....	.....	+2.20	72.1	62.3	15.3
	14	+0.60	60.9	37.8	26.3	+1.70	308.0	77.7	36.6	+0.50	57.4	25.2	24.8	+0.40	57.4	24.5	24.8	+0.40	57.4	24.5	24.8	+2.00	90.3	70.7	19.2
Control	..	-0.80	10.5	23.1	4.55	-0.90	11.2	42.0	1.33	-0.70	10.5	23.1	4.55	-0.70	10.5	23.1	4.55	-0.70	10.5	23.1	4.55	+1.30	3.5	20.3	0.74
Culture D	1	+0.50	51.1	24.5	22.1	+0.40	210.7	84.7	25.8	+0.50	51.7	22.4	22.1	.....	.....	.....	.....	.....	.....	.....	.....	+1.80	11.2	16.8	2.38
	3	+0.60	56.7	25.2	24.5	+1.50	273.7	69.3	32.1	+0.50	55.3	23.8	23.9	+0.50	54.6	25.2	23.6	+0.60	54.6	23.6	23.6	+1.70	14.0	32.2	2.98
	7	+0.50	57.4	28.7	24.8	+1.90	282.1	84.7	33.6	+0.50	56.7	24.5	24.5	.....	.....	.....	.....	.....	.....	.....	.....	+2.00	37.8	49.0	8.06
	10	+0.40	57.4	30.8	24.8	+2.20	307.3	76.3	36.5	+0.30	57.4	25.2	24.8	.....	.....	.....	.....	.....	.....	.....	.....	+2.30	72.8	50.4	15.5
	14	+0.40	58.1	32.9	25.1	+2.10	322.0	70.0	38.3	+0.40	57.4	28.0	24.8	+0.30	58.1	29.4	25.1	+0.70	55.3	27.3	23.9	+2.10	80.5	49.7	17.1
Control	..	-0.80	10.5	23.1	4.55	-0.90	11.2	42.0	1.33	-0.70	10.5	23.1	4.55	-0.70	10.5	23.1	4.55	-0.70	10.5	23.1	4.55	+1.30	3.5	20.3	0.74
Culture E	1	+0.50	51.1	22.1	22.1	+1.80	198.8	91.7	23.6	+0.50	49.7	21.5	21.5	.....	.....	.....	.....	.....	.....	.....	.....	+1.90	7.7	15.4	1.64
	3	+0.30	56.0	24.5	24.2	+1.80	296.8	96.0	35.3	+0.40	55.3	23.8	23.9	+0.30	53.9	23.8	23.3	+0.40	53.9	24.5	23.3	+2.10	9.8	29.4	2.08
	7	+0.30	58.1	25.2	25.1	+2.10	272.3	91.7	32.4	+0.30	57.4	24.5	24.8	.....	.....	.....	.....	.....	.....	.....	.....	+1.90	30.1	47.6	6.32
	10	+0.20	58.1	25.2	25.1	+2.20	312.9	77.7	37.2	+0.10	58.1	25.2	25.1	.....	.....	.....	.....	.....	.....	.....	.....	+1.80	50.4	56.0	10.7
	14	+0.10	58.1	25.2	25.1	+1.90	333.9	77.7	39.7	+0.10	58.8	25.9	25.4	+0.10	55.3	26.6	23.9	+0.10	56.0	25.2	24.2	+1.50	80.5	69.3	17.1
Control	..	-0.80	10.5	23.1	4.55	-0.90	11.2	42.0	1.33	-0.70	10.5	23.1	4.55	-0.70	10.5	23.1	4.55	-0.70	10.5	23.1	4.55	+1.50	3.5	20.3	0.74
Culture F	1	+0.20	52.5	22.4	22.7	+1.70	220.5	68.6	26.2	+0.90	51.8	21.0	22.4	.....	.....	.....	.....	.....	.....	.....	.....	+1.80	8.4	14.7	1.79
	3	+0.40	62.3	26.6	26.9	+1.70	310.8	70.0	37.0	+0.80	50.5	25.2	25.7	+0.40	60.2	26.6	26.0	+0.20	60.9	25.9	26.3	+1.80	10.5	26.6	2.23
	7	+0.30	63.7	27.3	27.5	+1.90	315.7	67.9	37.5	+0.60	60.2	28.7	26.0	.....	.....	.....	.....	.....	.....	.....	.....	+2.20	65.1	44.1	13.8
	10	+0.20	63.7	27.3	27.5	+2.00	318.5	70.7	37.9	+0.60	61.6	25.9	26.6	.....	.....	.....	.....	.....	.....	.....	.....	+2.30	70.7	46.6	15.0
	15	+0.10	62.3	26.6	26.9	+1.80	324.1	77.7	38.5	+0.50	62.3	25.2	26.9	+0.10	59.5	25.9	25.7	-0.10	60.2	26.6	26.0	+2.10	70.0	57.4	14.9

Reaction: -- = alkaline to neutral red; + = acid to neutral red; cc normal acid or alkali per 100 cc of medium  
Ammonia and amino nitrogen expressed in milligrams per 100 cc of culture medium.  
Control indicates initial composition of medium. Net results may be obtained by subtracting the control from the day's analysis.  
Amino nitrogen is corrected in each instance for ammonia.

microbe rather than cultural peculiarities have been the basis for identification.

The fermentation reactions are variously reported by different observers. Henry<sup>7</sup> states that glucose, levulose and maltose are fermented. Weinberg and Séguin<sup>1</sup> have found that the fermentative powers, if indeed any are demonstrable, proceed very slowly. They were unable to discover an evolution of gas or the formation of acid in any mediums. McIntosh and Feldes<sup>8</sup> state that the acid production is weak, but after seven days it is distinct in glucose, maltose and starch.

Bundles of gray-white crystals, which are acicular, form in the meat medium after several days' incubation. These crystals have been regarded as impure tyrosin.<sup>9</sup> They do not give the reactions of tyrosin, however. Their composition is still in doubt.

Six strains of *B. histolyticus* were studied in the series reported below. They were obtained from the following sources:

Culture, *B. histolyticus* A, from Dr. Holman.

Culture, *B. histolyticus* B, from Dr. Holman.

Culture, *B. histolyticus* C, from the National Research Council.

Culture, *B. histolyticus* D, from the National Research Council.

Culture, *B. histolyticus* E, from Dr. Meyer.

Culture, *B. histolyticus* F, from Dr. Meyer.

#### DISCUSSION

The biochemistry of *B. histolyticus* was studied by Wolf and Harris.<sup>10</sup> In a peptone water medium they found a measurable evolution of gas, amounting to about 70 c c in 4 days. There was also a considerable formation of ammonia, amounting to 24 mg. in the same time, and an equal increase in the amount of amino nitrogen. The reaction in terms of hydrogen-ion concentration remained practically the same during the entire period of incubation. In milk the gas formation was materially greater, amounting to about 300 c c for each liter of medium. It should be stated, however, that the time of incubation in the latter medium was 12 days. The formation of ammonia was decidedly greater in the milk medium which contained a greater total amount of protein, and of course a very material amount of highly

<sup>7</sup> Jour. Path. & Bacteriol., 1916, 21, p. 344.

<sup>8</sup> Med. Res. Committee, Special Report 12, 1917.

<sup>9</sup> Med. Res. Committee, Special Report 39, 1919.

<sup>10</sup> Jour. Path. & Bacteriol., 1918, 22, p. 1.

organized protein. In milk the ammonia production rose to the relatively considerable amount of 82 mg. per 100 c.c. of medium. The amino nitrogen content increased during the same incubation interval to 178 mg. per c.c. It appears probable from these analytic figures that *histolyticus* forms a soluble proteolytic enzyme which cleaves the milk proteins to amino acids and polypeptides greater in amount than the bacillus can utilize.

The strains of the organism identified as *B. histolyticus* in the series reported herein agree in salient metabolic features with the ones studied by Wolf and Harris.

In plain, glucose, lactose, saccharose, glycerol, starch and mannitol broths the nitrogenous changes, indicated by ammonia production and amino nitrogen accumulation, were quantitatively the same, suggesting that the presence of the non-nitrogenous sources of energy exercised no sparing action on the nitrogenous constituents of the medium. Furthermore, the cultures mentioned became slowly, but progressively acid, during the first few days of incubation. Later the titratable acidity decreased, suggesting that the gradual accumulation of basic products, typified by ammonia, exceeded in amount the acidic products which accumulated in the earlier days of growth, when the numbers of bacilli were increasing rather rapidly. At the same time there was a slow, indolent production of gas. Wolf and Harris<sup>10</sup> found this gas to consist chiefly of carbon dioxide (70 to 78%). The amino nitrogen formation is not large, proportionate to the ammonia formation in mediums containing peptone and meat extractives as the sole sources of nitrogen. It is not improbable that this may be explained in part by the relatively simple state of aggregation of the amino-acid complex which comprises the peptone molecule. The proteolytic enzyme of *B. histolyticus* might confidently be expected to have a less favorable field of activity in such relatively simple nitrogenous compounds. In opposition to this proportionately small accumulation of amino acid in the peptone mediums, the rapid and large amount of amino acids in mediums containing complex protein—gelatin and milk—stands in marked contrast. Here the amino acid accumulation (except in culture E, which failed to develop typically in milk) is strikingly characteristic. The bacilli are quite unable to utilize enough of the amino acids formed by the action of the proteolytic enzyme to reduce significantly the rapid accumulation of these substances, and the analytic figures show clearly how quickly the protein cleavage takes place.

The evolution of ammonia in milk is proportionately less than in gelatin. The action of *B. histolyticus* on the milk proteins is quantitatively different from that on gelatin. In both mediums, with the exception noted in the foregoing, the amino acid formation proceeds briskly, but in milk—for some reason for which an adequate explanation fails to present itself—the ammonia formation proceeds decidedly more slowly, and fails to approach that observed in the gelatin medium. It is a fact that the total nitrogen content of the gelatin medium used in these studies is about twice on the average that of the milk medium, but this explanation fails to account for the relatively exuberant formation in the former medium, because the amino-nitrogen content of both mediums during the entire course of the growth of the organism runs quantitatively nearly parallel.

#### SUMMARY

The strains of the anaerobic bacillus identified herein as *B. histolyticus* do not appear to be fermentative; the analytic tables fail to show any quantitative differences in mediums containing exactly the same nitrogenous constituents but differing in their carbohydrate content. In all mediums there is a progressive increase in titratable acidity for the first few days, followed by a slight recession of acidity as the basic products accumulate. There is coincidentally a slow, indolent evolution of gas, produced in approximately equal amounts in mediums containing peptone and meat extractives, irrespective of the carbohydrate content. In gelatin and milk, the gas production was quantitatively greater. The gaseous metabolism suggests that the origin of the gaseous products of growth is from the protein, and not influenced by the presence of any of the commoner carbohydrates. Glycerol also appears to exert no appreciable influence on the growth of the organism. This feature appears to be rather characteristic of *B. histolyticus*. Many anaerobes utilize glycerol.

On the other hand, the organism is actively proteolytic. The nitrogenous changes appear to be related quantitatively to the complexity of the nitrogenous constituents of the medium, at least in so far as gelatin and milk are concerned. The influence of other proteins was not tested.

The analytic tables indicate clearly that the organism described herein as *B. histolyticus* must be classed as of the obligately proteolytic group.

## BACILLUS PUTRIFICUS

## STUDY LVI

That group of anaerobic bacilli which is characterized morphologically by the formation of terminal spores greater in diameter than the parent rod has been a subject of controversy for several years. The isolation from infected wounds of warfare of several apparently distinct varieties or species exhibiting somewhat striking characteristics has redirected attention to them.

The first member of the group to be studied appears to have been *Bacillus putrificus*, described by Bienstock<sup>1</sup> in 1884. It was obtained from intestinal contents. It will be remembered that Escherich<sup>2</sup> described, but failed to isolate, his "Köpfchen bacillus" in the dejecta of young infants.

In the next two decades Klein,<sup>3</sup> Passini,<sup>4</sup> Rodella<sup>5</sup> and others obtained anaerobic bacilli of morphology similar to *B. putrificus* from feces, laboratory dust, soil and putrifying mixtures. These microbes were so similar in size, shape and sporulation, but so unlike in cultural characteristics that a somewhat acrimonious controversy arose among various observers regarding their identity. It seemed to be quite clear that at least two rather distinct varieties were obtained, differing in their apparent fermentative capacities, and Bienstock<sup>6</sup> published a description of a new organism exhibiting the morphology of *B. putrificus*, but apparently endowed with distinctly greater fermentative powers. The latter organism has been less thoroughly discussed than the preceding group.

The significance of *B. putrificus* to the bacteriologist resides rather in its theoretical relationship to that form of protein decomposition known as "putrefaction" than in its participation in human activities as an incitant of specific infection. In other words, *B. putrificus* seems to possess a chemical, rather than a pathogenic interest to the bacteriologist.

For many years the microbe was regarded as the type organism which induces that type of bacterial activity which Bienstock and,

<sup>1</sup> Ztschr. f. klin. Med., 1884, 8, p. 1; Arch. f. Hyg., 1889, 36, p. 335; 1901, 39, p. 390.

<sup>2</sup> Darmbakterien des Säuglings, 1886.

<sup>3</sup> Centralbl. f. Bakteriöl., 1899, 25, p. 278; 1901, 29, p. 991.

<sup>4</sup> Ztschr. f. Hyg. u. Infektionskr., 1905, 49, p. 135.

<sup>5</sup> Ann. Inst. Past., 1905, 19, p. 804.

<sup>6</sup> Ann. Inst. Past., 1906, 20, p. 497.



somewhat later, Rettger<sup>7</sup> termed "putrefaction." Two other anaerobic bacilli were regarded by Rettger as belonging to the group of true "putrefactive" bacteria, namely, *B. oedematis* (*Bacillus oedematis maligni*, or, as it is now termed, *Vibrio septique*), and *B. chauvœi* (the Rauschbrand bacillus, or *B. anthracis symptomatici*).<sup>8</sup>

The subsequent history of the conception of putrefaction, and the bacteria which are or were supposed to be the active incitants is interwoven with the gradual recognition of the difficulties attending the isolation and cultivation of cultures of anaerobic bacteria of unquestioned purity. Thus, Rettger's first studies on putrefaction<sup>7</sup> were recognized by him to have been vitiated by the presence of unrecognized contaminants in his cultural mediums.<sup>8, 9</sup> Even in 1912, however, before intensive studies of the bacilli of malignant edema and symptomatic anthrax were made with cultures of undoubted purity, Rettger still maintained that these two organisms were examples of the "putrefactive" type.<sup>10</sup>

More recent studies by Meyer,<sup>11</sup> and the striking work of Miss Robertson,<sup>12</sup> the Medical Research Committee,<sup>13</sup> and Weinberg and Séguin<sup>14</sup> have shown beyond reasonable doubt that the bacillus of malignant edema and the organism of symptomatic anthrax are practically devoid of proteolytic powers. In this respect they resemble the strongly fermentative anaerobic bacteria, such as the Welch bacillus.

Achalme<sup>15</sup> and others have studied cultures identified as *B. putrificus* with varied results, and comparatively recently Sturges and Rettger<sup>16</sup> have reopened the question of the identity of *B. putrificus* with observations apparently at variance with all previous work. They say: "All the strains of *B. putrificus* isolated by us exhibit a peculiar reluctance in undergoing development and in attacking the egg-meat medium in pure culture. The putrefaction is very much delayed and does not begin as a rule until twenty to thirty days after the beginning of incubation under anaerobic conditions. When once begun, however, the putrefaction is rapid and typical. This is in striking contrast with the other well-known putrefyers (*B. oedematis* and *B. chauvœi*) which usually begin to decompose the protein within a period of three to

<sup>7</sup> Am. Jour. Physiol., 1903, 8, p. 284.

<sup>8</sup> Rettger: Jour. Biol. Chem., 1906, 2, p. 85.

<sup>9</sup> Ibid., 1908, 4, p. 45.

<sup>10</sup> Ibid., 1912, 13, p. 341.

<sup>11</sup> Jour. Infect. Dis., 1915, 17, p. 458.

<sup>12</sup> Brit. Med. Jour., 1918, 1, p. 583.

<sup>13</sup> Special Report Series 39, 1919.

<sup>14</sup> La Gangrène Gazeuse, 1917.

<sup>15</sup> Ann. Inst. Past., 1902, 16, p. 633.

<sup>16</sup> Jour. Bacteriol., 1919, 4, p. 171.

four days. This delayed putrefaction of *B. putrificus* occurs only in pure cultures."

It is unusual for pure cultures of known anaerobic bacilli to exhibit a latent period of from two to three weeks in cultural mediums (where the gradual entrance of air would tend to create progressively unfavorable conditions for growth), followed by an apparently abrupt entrance into the vegetative state associated with rather intense chemical activity. Quantitative studies of the metabolism of such bacilli might throw some light on the nature of the process taking place, and possibly explain the cause or causes for the delayed growth. Whatever the explanation for this phenomenon may be, it is clear that the identity of *B. putrificus*, and even of the entire anaerobic plectridial group, with the exception of *B. tetani*, is still a matter of some doubt.

The important members of the plectridial group of anaerobes thus far described, comprise the following: *B. tetani*, *B. tertius*,<sup>17</sup> *B. tetanomorphus*,<sup>18</sup> (*B. pseudotetani*?), *B. cochlearius*,<sup>19</sup> *B. histolyticus*,<sup>20</sup> *B. putrificus*, and *B. paraputrificus*. It is not improbable that other members of this group, characterized morphologically by plectridial spores, may be isolated in the future.

The salient characteristics of those members of the group which appear to be sufficiently distinctive to identify a microbic entity, excluding *B. tetani*, *B. tertius*, *B. pseudotetani* and *B. histolyticus* (discussed in earlier communications of this series of studies), are inserted for purposes of orientation. In making this table, it is specifically understood that the final description of *B. putrificus* is yet to be elucidated. For historical reasons *B. putrificus* is specifically designated a proteolytic anaerobic bacillus, notwithstanding the fact that some observers have recently ascribed to the organism characteristics which would definitely remove it from the proteophilic group. It appears probable that the descriptions of these tabulated organisms, excluding *B. putrificus* and *B. paraputrificus*, are sufficiently well established to warrant at least their temporary acceptance as well defined microbic entities.<sup>18</sup> This would leave *B. putrificus* indeterminate but exhibiting historical characteristics not in opposition to those of two strains of anaerobic plectridial bacilli which have been isolated in this laboratory. One was obtained from feces (culture A), the other from a culture sent for diagnosis

<sup>17</sup> Henry: Jour. Path. & Bacteriol., 1916, 21, p. 344.

<sup>18</sup> McIntosh and Feldes: *Bacillus tetanomorphi*, Med. Res. Committee, Special Report Series 12, 1917.

<sup>19</sup> Type 3 C, McIntosh and Feldes, footnote 18.

<sup>20</sup> Weinberg and Séguin: Compt. rend. Soc. de biol., 1915, 78, p. 274; 1917, 80, p. 157.

from an unknown source (culture B). These two strains exhibit in common the ability to induce a gaseous fermentation in glucose and a rather noteworthy ability to produce evidence of proteolysis in mediums containing gelatin or milk proteins.

It should be emphasized that the proteolytic changes are decidedly less marked than those characteristic of *B. histolyticus* or of *B. sporogenes*, as evidenced by amino nitrogen formation. It is also wholly distinct from *B. tertius*,<sup>17</sup> both in its fermentative properties and in its ability to induce visible changes in milk and gelatin. It also differs from *B. pseudotetani*<sup>18</sup> in its biochemical properties.

TABLE A  
PLECTRIDIAL ANAEROBES

	<i>B. cochlearius</i>	<i>B. pseudotetani</i>	<i>B. putrificus</i> (?)	<i>B. paraputrificus</i>
Morphology.	Slender rod	Slender rod	Slender rod	Slender rod
Size.....	Same as tetanus	Same as tetanus	0.6-0.8 × 5-6 μ	Same as putrificus
Motility.....	Active	Active	Active	Active
Grouping....	Singly, pairs	Singly, pairs	Singly, pairs, rarely short chains	Same as putrificus
Capsule.....	Not demonstrated	Not demonstrated	Not demonstrated	Not demonstrated
Spore.....	Terminal, oval or spherical	Terminal, spherical	Terminal, oval or spherical	Terminal, oval or spherical
Stain.....	Gram + readily becomes Gram -	Gram + may become Gram -	Gram +*	Gram +*
Meat medium	Very little change	Pink color, some gas	Meat slowly digested	Gas, then digested
Gelatin.....	No softening	No softening	Liquefied	Liquefied
Milk.....	No visible change	No visible change	Peptonized	Gas and acid clot
Fermentation	None	Glucose and maltose	Glucose, maltose, glycerol, acid, some gas	Glucose, lactose, possibly other sugars

\* Old cultures may become irregularly gram-negative.

The relations of the cultural characteristics of the two strains under discussion to *B. cochlearius*, *B. pseudotetani*, and to the imperfectly described *B. paraputrificus*, are indicated in the tabulation (Table A). It is expressly understood that the designation "*Bacillus putrificus*" is to be construed as suggestive, rather than final. The organism agrees in essential details with that described as *B. putrificus* by the British Medical Research Committee.<sup>13</sup>

TABLE 1  
BACILLUS PUTRIFICUS

Control	Day	Plain				Gelatin				Glucose				Lactose				Saccharose				Milk			
		Reaction	Ammonia Nitrogen	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Amino Nitrogen	Percentage Ammonia to Total Nitrogen	Reaction	Ammonia Nitrogen	Amino Nitrogen	Percentage Ammonia to Total Nitrogen
Control	..	-0.80	12.6	21.0	5.61	-0.70	9.1	34.3	1.27	-0.60	12.6	21.0	5.61	-0.60	12.6	21.0	5.61	-0.80	12.6	21.0	5.61	+1.50	7.0	19.6	1.51
Culture A	1	+0.50	31.5	14.0	14.0	+0.90	32.9	23.8	4.61	+2.70	15.4	23.8	6.86	.....	.....	.....	.....	.....	.....	.....	.....	+2.50	9.1	17.5	1.96
	3	+1.00	54.6	24.5	24.3	+2.10	85.4	27.3	11.9	+3.20	17.5	23.9	7.80	+1.20	66.5	29.7	29.6	+1.10	51.8	19.6	23.1	+2.80	30.8	18.2	6.67
	6	+1.40	69.3	30.8	30.9	+2.40	121.8	29.4	17.0	+3.60	14.0	28.7	6.25	.....	.....	.....	.....	.....	.....	.....	.....	+3.00	77.7	30.8	16.8
	10	+1.50	77.7	34.8	34.6	+2.70	147.7	35.7	20.7	+3.30	9.8	30.8	4.37	.....	.....	.....	.....	.....	.....	.....	.....	+2.80	141.4	31.5	30.6
	14	+1.60	70.7	31.5	31.5	+3.00	157.5	37.8	22.0	+3.20	14.7	32.2	6.56	+1.40	78.4	35.0	34.9	+1.80	62.3	28.7	27.8	+2.90	140.0	31.5	30.3
Control	..	-0.80	12.6	22.4	5.61	-0.70	9.1	34.3	1.27	-0.60	12.6	21.0	5.61	-0.60	12.6	21.0	5.61	-0.80	12.6	21.0	5.61	+1.50	7.0	19.6	1.51
Culture B	1	+0.20	15.4	18.2	6.86	+0.10	16.8	28.0	2.35	+1.60	11.2	24.5	5.00	.....	.....	.....	.....	.....	.....	.....	.....	+2.60	22.4	22.4	5.07
	3	+0.90	56.0	16.8	24.9	+1.30	60.2	26.6	8.43	+3.30	11.9	24.5	5.30	+0.50	39.9	17.8	17.8	+1.70	44.8	20.3	20.0	+2.90	74.9	36.4	16.2
	6	+1.20	71.4	25.2	31.9	+2.90	142.1	36.4	19.9	+3.60	13.3	29.9	5.94	.....	.....	.....	.....	.....	.....	.....	.....	+3.10	137.9	34.8	29.8
	10	+1.30	70.7	28.7	31.5	+2.90	165.9	39.2	23.2	+3.60	14.0	31.5	6.25	.....	.....	.....	.....	.....	.....	.....	.....	+3.10	143.5	31.5	31.0
	14	+1.30	75.6	33.6	33.3	+2.90	182.0	42.7	25.5	+3.40	16.8	32.2	7.50	+1.00	72.1	32.2	32.1	+2.00	60.2	26.6	26.8	+3.20	143.5	34.5	31.0

Reaction: — = alkaline to neutral red; + = acid to neutral red; cc normal acid or alkali per 100 cc of medium.  
Ammonia and amino nitrogen expressed in milligrams per 100 cc of culture medium.

Control indicates initial composition of medium. Net results may be obtained by subtracting the control from the day's analysis.

Amino nitrogen is corrected in each instance for ammonia.

## DISCUSSION

The nitrogenous changes induced by the anaerobic plectridial bacilli, tentatively designated *B. putrificus*, are quite different from those described for other members of the terminal spore group, previously studied.<sup>21</sup> In plain, lactose, and saccharose mediums, there is a moderate formation of ammonia, and a relatively small accumulation of amino nitrogen which succeeds an initial decrease in these substances, containing  $\text{NH}_2$  groups which can be removed by the addition of liquor formaldehydi. The amount of nitrogenous change in these mediums, taken by themselves, would unquestionably place these organisms in a group characterized by moderate changes in protein mediums. Even after prolonged cultivation the amount of ammonia fails to exhibit an increase which would suggest a tendency toward marked proteolysis. The change in titratable acidity becomes slowly, but distinctly, acid, without however a marked departure from uninoculated controls. The

<sup>21</sup> See Kendall, Day and Walker: Studies XLVII, XLVIII, XLIX, and L, Jour. Infect. Dis., 1922, 30, pp. 167-177.

addition of glucose to the plain broth medium causes a distinct rise in titratable acidity; at the same time, an evolution of gas takes place which contrasts in amount and in the rate of formation with the indolent collection of bubbles of gas in the medium containing the same protein constituents, but not glucose. Also, the amino nitrogen fails to exhibit the decrease observed in the peptone mediums not containing this carbohydrate. The ammonia formation is distinctly less. The addition of glucose to peptone mediums, therefore, appears to exhibit that series of nitrogenous changes which are indicative of a sparing action of this carbohydrate for the protein constituents.

In gelatin and milk, the nitrogenous changes are distinctly greater, although they do not rise to the amounts formed by some of the active proteolytic anaerobic bacteria. In gelatin, the amount of ammonia formed is more than twice that produced in peptone mediums, and greater on the whole than that observed in milk. The amino nitrogen also falls below that of uninoculated controls during the earlier days of growth, but rises as the culture becomes older. Unlike *B. histolyticus*, the amino nitrogen content is less in gelatin than in milk, suggesting perhaps that the milk proteins are on the whole somewhat more utilizable for energy than the gelatin protein. The action of *B. putrificus*, judging from these results, is comparatively slight on peptones and meat extractives; this is in harmony with the more recent observations of a number of observers who have stated that *B. putrificus* is not a strongly proteolytic organism when grown in nutrient broth mediums. The action on gelatin also, while distinct, is on the whole not marked. Here again the contention that the organism is not of the marked proteolytic type would appear to be substantiated. In milk, the action on protein is distinctly more pronounced than on the other mediums studied. It is worthy of note that the initial decrease of amino acid and the formation of ammonia follows that characteristic of glucose broth rather than that observed in gelatin or peptone meat extractive mediums not containing a utilizable source of carbohydrate. After the first day or two, however, the increase in titratable acidity, the formation of ammonia and the accumulation of amino nitrogen is more in accord with the corresponding changes induced in mediums not containing a utilizable source of non-nitrogenous energy.<sup>22</sup> As milk contains about 0.1% of a substance appar-

<sup>22</sup> The tenth day flask of culture B in milk shows clearly that development has failed to take place at the normal rate. The value of the use of separate flasks for each day's analysis is shown by this culture, which is exceptional. As a rule, the flasks show a uniform "curve of growth" which is a valuable check on the purity of the culture.

ently glucose, the similarity of the growth curve for the first day in milk cultures is plausibly explained. After that time, or at least before the third day of growth was reached, the sugar of the medium was exhausted, and attack of the organisms was of necessity on the protein constituents of the medium.

#### SUMMARY

The organisms identified as *B. putrificus* are plectridial anaerobic bacilli of fairly definite proteolytic properties. On peptone-meat extractive mediums the nitrogenous transformations are moderate. There is a considerable formation of ammonia, an initial deficit in amino nitrogen, followed later by a progressive increase in amino nitrogen which usually exceeds by a small amount that of uninoculated controls. In peptone mediums the organism is not an active transformer of nitrogenous energy. The addition of glucose to these mediums reduces materially the formation of ammonia, and protects the amino nitrogen to a degree from bacterial transformation. In gelatin mediums the evidences of proteolytic activity are decidedly but not markedly greater. Gelatin is softened so that it will no longer solidify on cooling to the temperature of the icebox. The formation of ammonia is decidedly greater than in peptone medium, and there is a final concentration of amino nitrogen greater than in peptone mediums.

Milk proteins are somewhat more energetically attacked than gelatin in peptone proteins. Somewhat less ammonia, proportionately, is formed, and relatively more amino nitrogen is produced. The early hours of incubation in milk give rise to more acid, less ammonia, and a reduced diminution in amino nitrogen, suggesting an initial attack of the bacteria on the small amount of glucose in the milk. After this is used, the characteristic nitrogenous changes appear, suggestive of a greater cleavage of milk protein than gelatin protein, as evidenced by the greater amino acid accumulation.

*B. putrificus* appears to be an organism possessed of relatively limited but perfectly distinct proteolytic powers. Its fermentative properties are limited. The impression derived from a consideration of the respective proteolytic activities in peptone mediums, gelatin, and milk, in the order named, form a gradual progression in nitrogenous activity which would be misleading if each were considered by itself. It would appear that the milk proteins are most adapted to the nitrogenous requirements of *B. putrificus* in so far as these metabolic studies permit of comparison.