CONTRIBUTIONS TO THE BIOCHEMISTRY OF PATHOGENIC ANAEROBES.¹

IX.

THE BIOCHEMISTRY OF *BACILLUS ŒDEMATIENS*.

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IN the flora of "gas gangrene" wounds, *Bacillus œdematiens* occupies a position of almost equal importance with *B. welchii*, *B. sporogenes*, and *Vibrio septique*.

This organism, whose discovery we owe to Weinberg and Séguin in 1915, has been encountered in anaerobic wound infections by other investigators, and is characterised by the high toxicity of the products which it forms, and by the peculiar and typical gelatinous œdema which is produced either by the injection of toxins or of living cultures.

Biochemically, it appears to occupy a place intermediate between the saccharolytic *B. welchii*, which attacks saccharose, and the actively proteolytic *B. sporogenes* and *B. histolyticus*. Certain features of its growth show that it attacks proteins more actively than either *B. welchii* or *Vibrio septique*. It is said by Weinberg and Séguin (1917³) that in bouillon Martin a fœtid odour is obtained, especially in old cultures. The clot produced in milk is partly digested, but this only occurs after the lapse of some time.

According to Henry (1917¹), its saccharolytic power is limited, for it attacks only glucose, fructose, and maltose. Saccharose and lactose are unaffected. McIntosh (1917²) found that both saccharose and lactose are fermented with the production of considerable quantities of gas. The acid production is not great.

The lesions produced by the organism do not display the active and extensive tissue destruction found in infections with *B. sporogenes* and *B. histolyticus*. The action is in the direction of producing a gelatinous œdema infiltrated with small bubbles of gas. Lesions produced by pure cultures are never putrid.

¹ Received July 15, 1919. For earlier papers see this Journal, vols. xxi., xxii. and xxiii.

EXPERIMENTAL.

The series of experiments which follows was carried out on the same lines as previous work with other anaerobic micro-organisms. Weinberg insists on the necessity of the strictest anaerobiosis in dealing with *B. œdematiens*. The precautions which have been taken in previous work have proved ample, in dealing with this anaerobe, in producing an abundant and active growth. The flasks containing the media were evacuated to about 250 mm. of vacuum for twenty-four hours before inoculation, in order to test the tightness of all joints and the sterility of the medium. The contents of the flask were heated during this time to 38° C. in a thermostat. In this way practically all the dissolved air in the medium was expelled. Immediately after inoculation and the withdrawal of the control sample, the flask was evacuated as far as possible with a Fleuss pump, properly protected with drying traps. The medium boils vigorously, and in this way all dissolved air is removed. That the oxygen is removed is further shown by the analyses which are made of the gas produced at the end of the experiment.

Three strains of *B. œdematiens* were used in these experiments. They came from different sources. One strain, referred to as O.M., was provided by Dr. James McIntosh, of the London Hospital. The second strain, O.D., was given me by Captain H. Henry, R.A.M.C. This is the strain "Domange" with which so much work has been done in the study of *B. œdematiens* (Henry, 1917¹). One experiment was done with a second strain of *B. œdematiens*, sent me by Dr. McIntosh. It is referred to as O.M.M.

Fermentation of *B. œdematiens* in Peptone.

The peptone used was the ordinary tryptic broth employed in previous work. The inoculation was made into a medium having an initial reaction of $P_H = 6.83$.

B. œdematiens attacks tryptic broth with the formation of gas and ammonia, but the reaction is relatively slow. From 1500 c.c. of medium only 25 c.c. of gas are formed in twenty-four hours, and at the end of 192 hours only 140 c.c. of gas were evolved. In an intermediate time, namely, 48 hours, the amount was half that obtained from *V. septique* under similar conditions. After seventy-two hours gas was evolved with great slowness. Amino-acid nitrogen and ammonia nitrogen are both formed in some quantity, but they, together with the volatile acids, seem to reach a maximum in the period between the third and fifth days. From this time on, the values obtained grow less. This is apparently accompanied by a small reversal in reaction; the hydrogen-ion concentration at the 150th hour being $P_H = 6.73$, while at the 168th hour the medium became more alkaline, $P_H = 6.83$. The last sample was not tested for reaction.

Fermentation of *B. œdematiens* in Glucose Peptone.

Three experiments were performed with three different strains of this anaerobe. The peptone contained 2 per cent. of glucose. The gas production

from the three strains varied considerably. Excluding Experiment No. 3, in which observations were only made to the twenty-third hour, the experiments give as final readings 813 and 301 c.c. of gas respectively. The other components of the resulting fermentation show a similar difference. In the case where the gas formation was large, there was a very decided increase in amino-acid formation. The ammonia formation was not large in any of these fermentations. The production of volatile acids was only considerable in the experiment where gas was evolved in quantity.

The final reaction was only determined in the experiment with large gas production. The hydrogen exponent fell to $P_H = 5.23$, showing a more alkaline final reaction than is observed with saccharolytic anaerobes under similar conditions.

Fermentation of *B. œdematiens* in Milk.

B. œdematiens attacks milk vigorously, with the formation of very considerable quantities of gas. The reaction takes place quickly, for inside of twenty-four hours 150 c.c. of gas were formed from 1500 c.c. of medium. In 150 hours 755 c.c. of gas were formed. With this gas production there is a consumption of lactose, which, measured as loss of reducing substance, amounts to 6.7 grms. of sugar per litre of medium. On long incubation there is apparently a hydrolysis of the disaccharide, for the reducing power of the medium increases, a circumstance which Wolf and Harris (1917⁵) first observed with *B. sporogenes*. The attack on protein, as measured both by the ammonia formation and the amino-acid formation, is very slight. Only 8.5 mgrms. of amino-acid nitrogen per 100 c.c. of medium and 1.9 mgrm. of ammonia were formed at the end of the experiment.

There is a notable amount of volatile acid formed, and as a result, owing to the lack of basic groups set free, the reaction falls from an initial reaction of $P_H = 6.34$ to $P_H = 4.28$ at the 122nd hour. The main features, therefore, of the action of *B. œdematiens* on milk are the large gas formation with sugar consumption, and the increase in acidity. It will be observed that vigorous gas production is taking place at a much higher acidity than is observed with *B. welchii*.

Fermentation of *B. œdematiens* in Cooked Meat.

The naked-eye appearance of cooked-meat cultures of *B. œdematiens* is characteristic, and is not simulated by any other of the pathogenic anaerobes. In the course of time the volume of meat diminishes, and the remaining undigested protein becomes yellowish-grey in colour, the yellowish colour predominating in the upper layers. It is obvious that the organism has a considerable proteolytic power.

The proteolysis produced by an active culture of *B. œdematiens* is well shown in Experiment 6. The gas production is also well marked.

In the first six and a half hours 114 c.c. of gas were produced per litre of medium, and in ninety-eight hours 544 c.c. were evolved. With this an active proteolysis takes place. In 215 hours 81.3 mgrms. of amino-acid nitrogen were formed. The amount of ammonia produced is not considerable. The deaminating properties of the bacillus seem to be slight when grown in this medium.

The volatile-acid production occupies a middle place when compared with anaerobes previously examined. It is somewhat larger than that obtained with *V. septique*, but is much smaller than what is formed by the truly proteolytic organisms *B. sporogenes* and *B. histolyticus*. The effect of acid formation on the reaction of the medium is pronounced, the acid reaction increasing from $P_H = 6.74$ to $P_H = 5.52$.

The gas evolved consisted of 52·1 per cent. of carbon dioxide and 47·9 per cent. of hydrogen.

The second experiment was performed with the McIntosh strain. The concentration of the protein in the medium was higher. The evolution of gas was somewhat slower in starting, but the eventual amount produced was comparable with the preceding fermentation. There is in this experiment a larger amount of ammonia produced and less amino-acid formed. The volatile acid production is similar to the first experiment. As a result, there is a smaller rise in hydrogen-ion concentration, the final reaction of the medium at the end of eighty-five hours being $P_H=5\cdot73$. The composition of the gas shows it to be over 9 per cent. richer in carbon dioxide, the analysis giving 60·4 per cent. of this gas.

SUMMARY.

B. cedematiens possesses characteristics which are found in both the proteolytic and saccharolytic organisms previously examined. The saccharolytic characters are greatly predominant. In glucose and lactose-containing media it is an efficient gas producer. Lactose is consumed in considerable quantity. It also has the property of hydrolysing this sugar.

Large quantities of gas are produced in cooked-meat media containing no free carbohydrates. Notable quantities of amino-acids and ammonia may also be found.

PROTOCOLS OF EXPERIMENTS.

Experiment 1.

DATA.

1500 c.c. of tryptic broth medium sterilised at 120° for sixty minutes; inoculated with O.D. (14-hour-old cooked-meat culture); incubated at 38° C.

Initial Composition of Medium—

Total nitrogen	0·398 per cent.
Ammonia-nitrogen	20·6 mgrms. per 100 c.c.
Amino-acid nitrogen	102·0 „ „ 100 „
Volatile acids	5·6 c.c. N/10 alkali „ 100 „

Gas Production—

Volume of gas per litre of medium (192 hours)	140·7 c.c.
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Ammonia Formation—

Final content of ammonia-nitrogen	35·2 mgrms. per 100 c.c.
Therefore 17·6 mgrms. of ammonia-nitrogen were formed per 100 c.c.	

Amino-acid Formation—

Final content of amino-acid nitrogen	117·0 mgrms. per 100 c.c.
Therefore 15 mgrms. of amino-acid nitrogen were formed per 100 c.c.	
Amount of total nitrogen present as ammonia and amino-acid nitrogen at the end of fermentation	152·2 mgrms.
Therefore 7·4 per cent. of the total nitrogen was transformed into ammonia and amino-acid nitrogen.	

Volatile Acid Formation—

Total amount of volatile acids present at the end of fermentation	18·4 c.c. N/10 alkali per 100 c.c.
Therefore amount of volatile acids formed during fermentation is 12·8 c.c. N/10 alkali.	

Hydrogen-ion Concentration—

Initial	.	.	.	.	.	.	P _H =6·83
Final	.	.	.	.	.	.	P _H =6·83

Table I.

B. OEDEMATIENS ON PEPTONE WATER.

Number of Sample.	Time in Hours.	Gas. C.c. per Litre.	Content of Amino-acid Nitrogen. Mgrms. per 100 c.c.	Amount of Amino-acid Nitrogen formed. Mgrms. per 100 c.c.	Content of Ammonia-Nitrogen. Mgrms. per 100 c.c.	Amount of Ammonia-Nitrogen formed. Mgrms. per 100 c.c.	Content of Volatile Acids. C.c. N/10 Solution per 100 c.c.	Volatile Acids formed. C.c. N/10 Solution per 100 c.c.	P _H .
1	...	...	102	...	20·6	...	5·6	...	6·83
2	24	25·6	111	9	30·6	10·0	14·4	8·8	...
3	48	65·9	117	15	36·0	15·4	21·8	16·2	...
4	72	104·7	119	17	38·9	18·3	27·5	21·9	...
5	122	111·7	115	13	38·4	17·8	19·4	13·8	...
6	150	111·7	115	13	38·2	17·6	18·8	13·2	6·73
7	168	114·7	113	11	38·4	17·8	20·1	14·5	6·83
8	192	140·7	117	15	35·2	14·6	18·4	12·8	...

Experiment 2.

DATA.

1500 c.c. of glucose-peptone medium sterilised at 120° for sixty minutes; inoculated with O.D. 6 (14-hour-old glucose peptone culture); incubated at 38° C.

Initial Composition of Medium—

Total nitrogen	.	.	.	.	.	0·368 per cent.
Ammonia-nitrogen	.	.	.	.	.	23·6 mgrms. per 100 c.c.
Amino-acid nitrogen	.	.	.	.	.	118 „ „ 100 „
Volatile acids	.	.	.	.	.	9·1 c.c. N/10 alkali „ 100 „

Gas Production—

Volume of gas per litre of medium (twenty-five hours)	.	813·6 c.c.
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Ammonia Formation—

Final content of ammonia-nitrogen	.	.	.	24·2 mgrms. per 100 c.c.
Therefore 0·6 mgrms. of ammonia-nitrogen were formed per 100 c.c.				

Amino-acid Formation—

Final content of amino-acid nitrogen	.	.	.	188 mgrms. per 100 c.c.
Therefore 70 mgrms. of amino-acid nitrogen were formed per 100 c.c.				

Amount of total nitrogen present as ammonia and amino-acid nitrogen at the end of fermentation . . . 212·2 mgrms.
Therefore 19·2 per cent. of the total nitrogen was transformed into ammonia and amino-acid nitrogen.

Volatile Acid Formation—

Total amount of volatile acids present at the end of
fermentation 29·4 c.c. N/10 alkali per 100 c.c.
Therefore amount of volatile acids formed during fermentation
is 20·3 c.c. N/10 alkali.

Hydrogen-ion Concentration—

Initial $P_H = 6·37$
Final $P_H = 5·23$

Table II.

B. OEDEMATIENS ON GLUCOSE PEPTONE.

Number of Sample.	Time in Hours.	Gas. C.c. per Litre.	Content of Amino-acid Nitrogen. Mgrms. per 100 c.c.	Amount of Amino-acid Nitrogen formed. Mgrms. per 100 c.c.	Content of Ammonia-Nitrogen. Mgrms. per 100 c.c.	Amount of Ammonia-Nitrogen formed. Mgrms. per 100 c.c.	Content of Volatile Acids. C.c. N/10 Solution per 100 c.c.	Volatile Acids formed. C.c. N/10 Solution per 100 c.c.	P_H .
1	...	...	118	...	23·6	...	9·1	...	6·37
2	8	9·3	120	2	25·6	2·0	10·9	1·8	6·27
3	23	256·0	112	—	24·0	0·4	22·5	13·4	5·23 +
4	25	813·6	109	—	22·8	—	23·1	14·0	5·23
5	28	...	113	—	26·1	2·5	24·8	15·7	5·23
6	31	...	116	—	24·9	1·3	24·7	15·6	5·23
7	48	...	120	2	24·9	1·3	26·0	16·9	5·23
8	96	...	127	9	24·9	1·3	30·2	21·1	5·23
9	120	...	188?	70?	24·2	0·6	29·4	20·3	...
10	675	...	126	8	26·2	2·6	30·8	21·7	...

Experiment 3.

DATA.

1500 c.c. of glucose-peptone medium sterilised at 120° for sixty minutes; inoculated with O.M. 7 (14-hour-old glucose peptone culture); incubated at 38° C.

Initial Composition of Medium—

Total nitrogen 0·325 per cent.
Ammonia-nitrogen 21·0 mgrms. per 100 c.c.
Amino-acid nitrogen 117·0 „ „ 100 „
Volatile acids 60 c.c. N/10 alkali „ „ 100 „

Gas Production—

Volume of gas per litre of medium (twenty-three hours) 145·8 c.c.

Ammonia Formation—

Final content of ammonia-nitrogen 25·5 mgrms. per 100 c.c.
Therefore 4·5 mgrms. of ammonia-nitrogen were formed per 100 c.c.

Amino-acid Formation—

Final content of amino-acid nitrogen 124 mgrms. per 100 c.c.
 Therefore 7 mgrms. of amino-acid nitrogen were formed per 100 c.c.

Amount of total nitrogen present as ammonia and amino-
 acid nitrogen at the end of fermentation 149.5 mgrms.
 Therefore 3.5 per cent. of the total nitrogen was transformed
 into ammonia and amino-acid nitrogen.

Volatile Acid Formation—

Total amount of volatile acids present at the end of fer-
 mentation 9.2 c.c. N/10 alkali per 100 c.c.
 Therefore amount of volatile acids formed during fermentation
 is 3.2 c.c. N/10 alkali.

Hydrogen-ion Concentration—

Initial $P_H = 6.30$
 Final $P_H = 5.23 ?$

Table III.

B. OEDEMATIENS ON GLUCOSE PEPTONE.

Num- ber of Sample.	Time in Hours.	Gas. C.c. per Litre.	Content of Amino- acid Nitrogen. Mgrms. per 100 c.c.	Amount of Amino- acid Nitrogen formed. per 100 c.c.	Content of Ammonia- Nitrogen. Mgrms. per 100 c.c.	Amount of Ammonia- Nitrogen formed. Mgrms. per 100 c.c.	Content of Volatile Acids. C.c. N/10 Solution per 100 c.c.	Volatile Acids formed. C.c. N/10 Solution per 100 c.c.	P_H .
1	...	...	117	—	21.0	...	6.0	...	6.30
2	2	11.8	107	—	22.8	1.8	6.4	0.4	5.25
3	8	135.8	108	—	23.0	2.0	7.3	1.3	...
4	23	145.8	112	—	23.3	2.3	11.7	5.7	...
5	31	—	115	—	23.4	2.4	14.4	8.4	...
6	48	—	117	—	23.7	2.7	16.9	10.9	...
7	96	—	121	4	23.6	2.6	17.7	11.7	...
8	120	...	124	7	25.5	4.5	9.2	3.2	...

Experiment 4.**DATA.**

1500 c.c. of glucose-peptone medium, sterilised at 120° for sixty minutes; inoculated with
 O.M.M. (14-hour-old cooked-meat culture); incubated at 38° C.

Initial Composition of Medium—

Total nitrogen 0.334 per cent.
 Ammonia-nitrogen 17.4 mgrms. per 100 c.c.
 Amino-acid nitrogen 112 „ „ 100 „
 Volatile acids 4.4 c.c. N/10 alkali „ 100 „

Gas Production—

Volume of gas per litre of medium (126 hours) 301 c.c.

Ammonia Formation—

Final content of ammonia-nitrogen 31.7 mgrms. per 100 c.c.
 Therefore 13.7 mgrms. of ammonia-nitrogen were formed per 100 c.c.

Amino-acid Formation—

Final content of amino-acid nitrogen 107 mgrms. per 100 c.c.
 Therefore 0 mgrms. of amino-acid nitrogen were formed per 100 c.c.¹

Amount of total nitrogen present as ammonia and amino-
 acid nitrogen at the end of fermentation 138 mgrms.
 Therefore 3.6 per cent. of the total nitrogen was transformed into
 ammonia and amino-acid nitrogen.

Volatile Acid Formation—

Total amount of volatile acids present at the end of fermenta-
 tion 6.0 c.c. N/10 alkali per 100 c.c.
 Therefore amount of volatile acids formed during fermentation
 is 1.6 c.c. N/10 alkali.

Hydrogen-ion Concentration—

Initial $P_H = 6.90$.

Table IV.

B. ŒDEMATIENS ON GLUCOSE PEPTONE.

Number of Sample.	Time in Hours.	Gas. C.c. per Litre.	Content of Amino-acid Nitrogen. Mgrms. per 100 c.c.	Amount of Amino-acid Nitrogen formed. Mgrms. per 100 c.c.	Content of Ammonia-Nitrogen. Mgrms. per 100 c.c.	Amount of Ammonia-Nitrogen formed. Mgrms. per 100 c.c.	Content of Volatile Acids. C.c. N/10 Solution per 100 c.c.	Volatile Acids formed. C.c. N/10 Solution per 100 c.c.	P_H .
1	...	...	112	...	17.4	...	4.4	...	6.90
2	5.3	33	119	7	20.3	2.9	6.4	2.0	...
3	23.0	220	133	21	29.5	12.1	9.4	5.0	...
4	28.7	235	168	56	31.1	13.7	8.6	4.2	...
5	48.2	...	129	17	31.7	14.3	7.3	2.9	...
6	97.5	266	112	0	31.2	13.8	6.4	2.0	...
7	126.0	301	107	0	31.1	13.7	6.0	1.6	...

Experiment 5.**DATA.**

1500 c.c. of skimmed milk medium; sterilised at 120° for sixty minutes; inoculated with O.M. 5 (14-hour-old milk culture); incubated at 38° C.

Initial Composition of Medium—

Total nitrogen 0.480 per cent.
 Ammonia-nitrogen 11.6 mgrms. per 100 c.c.
 Amino-acid nitrogen 8.4 " " 100 "
 Volatile acids 10.0 c.c. N/10 alkali " 100 "

¹ Amino-nitrogen was formed, but was used up at end of experiment.

Experiment 6.

DATA.

1500 c.c. of cooked-meat medium; sterilised at 120° for sixty minutes; inoculated with O.D. 6 (14-hour-old cooked-meat culture); incubated at 38° C.

Initial Composition of Medium—

Total nitrogen	.	.	.	.	.	0.399 per cent.
Ammonia-nitrogen	.	.	.	.	.	19.4 mgrms. per 100 c.c.
Amino-acid nitrogen	.	.	.	.	.	22.7 „ „ 100 „
Volatile acids	.	.	.	.	.	14.7 c.c. N/10 alkali „ 100 „

Gas Production—

Volume of gas per litre of medium (215 hours)	.	.	544 c.c.
Carbon dioxide	.	.	60.4 per cent.
Hydrogen	.	.	39.6 „

Ammonia Formation—

Final content of ammonia-nitrogen	.	.	33.9 mgrms. per 100 c.c.
Therefore 14.5 mgrms. of ammonia-nitrogen were formed per 100 c.c.			

Amino-acid Formation—

Final content of amino-acid nitrogen	.	.	104 mgrms. per 100 c.c.
Therefore 81.3 mgrms. of amino-acid nitrogen were formed per 100 c.c.			

Amount of total nitrogen present as ammonia and amino-acid nitrogen at the end of fermentation . . . 137.9 mgrms.

Therefore 10.5 per cent. of the total nitrogen was transformed into ammonia and amino-acid nitrogen.

Volatile Acid Formation—

Total amount of volatile acids present at end of fermentation	.	.	55.3 c.c. N/10 alkali per 100 c.c.
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Therefore amount of volatile acids formed during fermentation is 40.7 c.c. N/10 alkali per 100 c.c.

Hydrogen-ion Concentration—

Initial	.	.	.	.	.	P _H = 6.74.
Final	.	.	.	.	.	P _H = 5.52.

Table VI.

B. OEDEMATIENS ON COOKED-MEAT MEDIUM.

Number of Sample.	Time in Hours.	Gas. C.c. per Litre.	Content of Amino-acid Nitrogen. Mgrms. per 100 c.c.	Amount of Amino-acid Nitrogen formed. Mgrms. per 100 c.c.	Content of Ammonia-Nitrogen. Mgrms. per 100 c.c.	Amount of Ammonia-Nitrogen formed. Mgrms. per 100 c.c.	Content of Volatile Acids. C.c. N/10 Solution per 100 c.c.	Volatile Acids formed. C.c. N/10 Solution per 100 c.c.	P _H .
1	...	...	22.7	...	19.4	...	14.7	...	6.74
2	6.5	114	24.7	2.0	20.6	1.2	38.3	23.6	5.82
3	8.5	282	33.5	10.8	21.2	1.8	76.8	32.1	5.82
4	10.75	385	44.3	21.6	22.4	3.0	49.5	34.8	5.78
5	24.0	466	57.6	34.9	20.4	1.0	51.8	37.1	5.80
6	49.0	518	61.9	39.2	25.3	5.9	50.8	36.1	5.56
7	79.0	538	81.0	58.3	27.9	8.5	51.1	36.4	5.54
8	98.0	544	89.8	67.1	28.7	9.3	51.7	37.0	5.52
9	215	...	104.0	81.3	33.9	14.5	55.3	40.6	...

Experiment 7.

DATA.

1500 c.c. of cooked-meat medium; sterilised at 120° for sixty minutes; inoculated with O.M. 7 (14-hour-old cooked-meat culture); incubated at 38° C.

Initial Composition of Medium—

Total nitrogen	0.95 per cent.
Ammonia-nitrogen	20.9 mgrms. per 100 c.c.
Amino-acid nitrogen	7.1 „ „ 100 „
Volatile acids	10.4 c.c. N/10 alkali „ 100 „

Gas Production—

Volume of gas per litre of medium (82.2 hours)	478 c.c.
Carbon dioxide	70.0 per cent.
Hydrogen	30.0 per cent.

Ammonia Formation—

Final content of ammonia-nitrogen	55.0 mgrms. per 100 c.c.
Therefore 34.1 mgrms. of ammonia-nitrogen were formed per 100 c.c.	

Amino-acid Formation—

Final content of amino-acid nitrogen	47.1 mgrms. per 100 c.c.
Therefore 40.0 mgrms. of amino-acid nitrogen were formed per 100 c.c.	
Amount of total nitrogen present as ammonia and amino-acid nitrogen at the end of fermentation	102.1 mgrms.
Therefore 7.5 per cent. of the total nitrogen was transformed into ammonia and amino-acid nitrogen.	

Volatile Acid Formation—

Total amount of volatile acids present at the end of fermentation	54.5 c.c. N/10 alkali per 100 c.c.
Therefore amount of volatile acids formed during fermentation is 44.1 c.c. N/10 alkali per 100 c.c.	

Hydrogen-ion Concentration—

Initial	P _H =6.74.
Final	P _H =5.73.

Table VII.

B. ŒDEMATIENS ON COOKED-MEAT MEDIUM.

Number of Sample.	Time in Hours.	Gas. C.c. per Litre.	Content of Amino-acid Nitrogen. Mgrms. per 100 c.c.	Amount of Amino-acid Nitrogen formed. Mgrms. per 100 c.c.	Content of Ammonia-Nitrogen. Mgrms. per 100 c.c.	Amount of Ammonia-Nitrogen formed. Mgrms. per 100 c.c.	Content of Volatile Acids. C.c. N/10 Solution per 100 c.c.	Volatile Acids formed. C.c. N/10 Solution per 100 c.c.	P _H .
1	0	...	7.1	...	20.9	...	10.4	...	6.74
2	7.25	34	9.3	2.2	20.9	0	16.5	6.1	6.19
3	9.25	112	10.1	3.0	20.8	0	45.6	35.2	5.96
4	10.75	176	12.7	5.6	21.8	0.9	47.3	36.9	5.94
5	24.0	346	17.8	10.7	25.1	4.2	49.8	39.4	5.84
6	49.0	417	22.6	15.5	28.5	7.6	49.7	39.3	5.65
7	67.25	442	27.7	20.6	31.5	10.6	50.0	39.6	5.73
8	85.25	478	28.8	21.7	35.1	14.2	50.6	40.2	5.73
9	200.25	...	47.1	40.0	55.0	34.1	54.5	44.1	...

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Table VIII.—*BACILLUS ŒDEMATIENS*.—

Experiment.	Medium.	INITIAL COMPOSITION OF MEDIUM.						Laboratory Number of Cultures used as Seed.	Number of Hours' Fermentation.
		Nitrogenous Matter.				Volatile Acids. C.c. N/10 Solution per 100 c.c.	Initial Reaction Ph.		
		Total Nitrogen. Mgrms. per 100 c.c.	Amino-Nitrogen. Mgrms. per 100 c.c.	Ammonia-Nitrogen. Mgrms. per 100 c.c.	Proportion of Total Nitrogen present as NH ₂ and NH ₃ .				
1	Peptone	398	102.0	20.6	30.8	5.6	6.3	O.D. 1	192
2	Glucose peptone	368	118.0	23.6	33.3	9.1	6.37	O.D. 6	675
3	„	325	117.0	21.0	42.4	6.0	6.30	O.M. 7	120
4	„	334	112.0	17.4	38.9	4.4	6.90	O.M.M.1	126
5	Milk	480	8.4	11.6	4.1	10.0	6.34	O.D. 5	192
6	Cooked meat	399	22.7	19.4	10.5	14.7	6.74	O.D. 6	215
7	„	950	7.1	20.9	2.9	10.4	6.74	O.M. 7	200

SUMMARY OF EXPERIMENTS.

FINAL COMPOSITION OF MEDIUM.									Final Reaction Ph.	GAS.		
Nitrogenous Matter.						Volatile Acids.				Volume of Gas. C.c. per Litre.	CO ₂ per cent.	H ₂ per cent.
Amino-Nitrogen.		Ammonia-Nitrogen.		Proportion of Total Nitrogen present as NH ₂ and NH ₃ .	Proportion of Total Nitrogen transformed during Fermentation.	Content. C.c. N/10 Solution per 100 c.c.	Amount formed. C.c. N/10 Solution per 100 c.c.					
Content. Mgrms. per 100 c.c.	Amount formed. Mgrms. per 100 c.c.	Content. Mgrms. per 100 c.c.	Amount formed. Mgrms. per 100 c.c.									
117.0	15.0	35.2	14.6	38.2	6.4	18.4	12.8	6.83	140.7	..	..	
188.0	70.0	24.2	0.6	57.6	19.2	29.4	20.3	5.23	813.6	..	..	
124.0	7.0	25.5	4.5	46.0	3.4	9.2	3.2	5.23?	145.8 ¹	..	..	
107.0	-	31.1	13.7	42.3	3.6	6.0	1.6	..	301.0	..	..	
16.9	8.5	13.5	1.9	6.3	2.2	34.2	24.2	4.28	755.0	52.1	47.9	
104.0	81.3	33.9	14.5	34.5	24.0	55.3	40.7	5.52	544.0	60.4	39.6	
47.1	40.0	55.0	34.1	10.7	7.8	54.5	44.1	5.73	478.0	70.0	30.0	

¹ At 3 hours.