A STUDY OF SOME GAS-PRODUCING BACTERIA.

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THE manifold interests connected with micro-organisms, because of their causal relations to certain diseases of man and also of the lower animals, and the part they play in many important economic problems have stimulated much investigation by physicians and biologists. The field is, however, very great and the explorations have but just begun.

There are many questions connected with this subject that are yet unsettled and many that have not as yet been touched upon. The physician in studying micro-organisms has always before him the consideration of their relation to the disease in question. Other phases of the life of these germs are not at all considered by these investigators, thus leaving the important questions of classification, physiology and the chemistry of their life and development to the botanist and chemist. The purpose of this paper is to make a small contribution to a phase of the chemical side of this question, namely, to a study of the gases produced during the development and growth of some micro-organisms.

Although the study of the products of chemical decomposition formed by micro-organisms has not been very extensive or very thorough, yet much has been learned in a qualitative way. The substances produced by bacteria are quite numerous, including solids, liquids and gases. The same products are often produced by different organisms in varying proportions.

Among the solids produced are the ptomaines, indol, skatol, leucine, tryrosine, succinic and malic acids, etc. The liquid products include alcohol, acetic and lactic acids. The gases formed are quite numerous and include hydrogen sulphide, ammonia, carbon dioxide, hydrogen and methane. In cases in which ammonia and hydrogen sulphide are produced simultaneously, they unite and form ammonium sulphide.

The importance of the study of these compounds, both qualitatively and quantitatively, is evident when the character of such products as are included under the general term ptomaines and leucomaines, also tuberculin, antitoxine, etc., are consid-

ered. A knowledge of the gaseous products and the conditions under which they are formed is often of great service to the biologist in identifying different species. This is well illustrated by a condensed statement taken from on article by Dr. McWeemey in "Modern Medicine and Bacteriological Review," Vol. **3,** August, 1894.

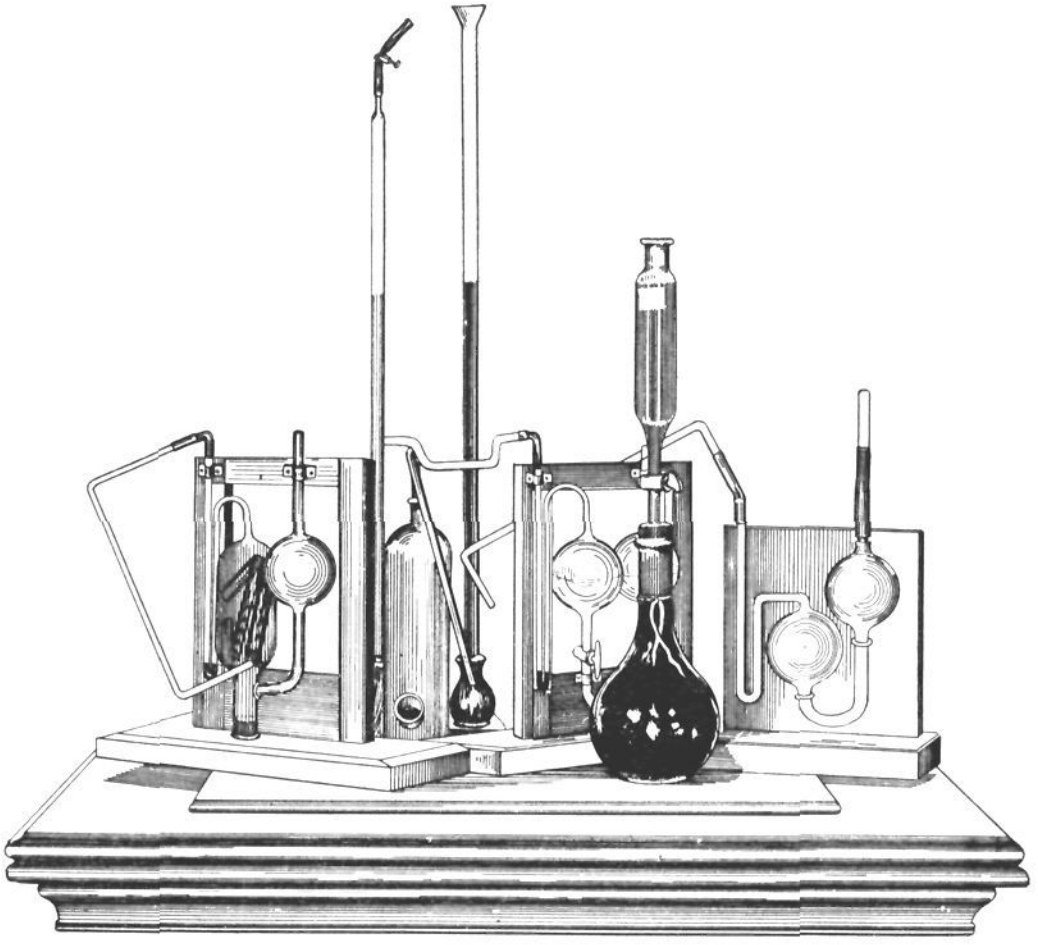
Dr. McWeeney says in this article that he made a study of the microbic cause of an epidemic of typhoid fever, which had recently occurred in the village of Waterford, England, using the method of Pariettis, Globis, and others. He identified the microbes as those of Eberths, (that causing typhoid fever) while the fermentation test of Dr. Theobald Smith, of Washington, indicated on the contrary that the bacillus was not that of Eberth, but was the bacillus coli, since it produced decomposition of lactose, although not quite so freely as another specimen of bacillus coli with which it is compared. The bacillus of Eberth's did not, however, produce decomposition of lactose as does the bacillus coli.

It is a well known fact that micro-organisms, when surrounded by the conditions favorable to their growth, namely, a proper food supply, moisture, favorable atmospheric conditions, and temperature, develop very rapidly for a time. However, after a certain period the rate of development diminishes until finally it ceases entirely, although there may be a large supply of food material still unused and the general conditions have not changed. For example, the saccharomyces produces alcohol from sugar until about fifteen per cent. of the media becomes alcohol, when action practically ceases. A twenty per cent. solution of alcohol is antiseptic. Many illustrations for the effect on the growth of bacterial forms might be adduced, but they are too familiar facts to be repeated here.

In a study made by the authors an attempt was made to accurately estimate the constituents of the mixed gaseous products by a variety of bacteria. Hempel's apparatus was used for the estimation of the gases. The pieces employed are shown in the cut that accompanies this article.[1] The culture flask is the only apparatus that needs description. It consists of a half liter flask

[1] See Hempel's "Gas Analysis" for description.

closed with a three-holed rubber stopper, through one of the openings of which is passed the stem of a 100 cc. separatory funnel until it nearly reaches the bottom of the flask. Into the



second opening is inserted a fine capillary tube, bent at right angles, which serves to conduct the gas to the mercury gasometer. The third opening serves for the thermometer when temperature determinations are made. The flask is connected with the mercury gas-holder when ready for the connection of the gases.

The separatory funnel was used for inoculating the medium. The method of procedure is as follows: The flask is filled nearly full of the food medium which had been properly sterilized. The separatory funnel, which was filled about two-thirds full of the sterilized medium, was now inserted and the whole resterilized.

The stopper was next crowded in and the stopcock opened. The medium in the funnel was allowed to run into and fill the small vacant space below the stopper and force the liquid through the capillary tube until the latter is filled over to the mercury. The stopcock was then closed and the apparatus was ready for the collection of the gases. From the gas-holder the gas was transferred to the burette and the analysis carried on in the usual manner, except that mercury was used in the burettes instead of water.

PREPARATION OF THE MEDIA.

The fluid in all cases was peptone bouillon containing either glucose, lactose, or cane sugar. The bouillon was prepared by using either 250 grams of finely chopped lean beef, or five grams of Liebig's extract. After the meat was thoroughly cooked it was filtered, and to the filtrate was added five grams of salt, twenty grams of sugar and ten grams of dried peptone. It was then made up to a liter. The medium was now put in a flask and separatory funnel as before directed and sterilized for three successive days in a Kock's steam sterilizer, when it was ready for use. The Liebig's extract was treated in the same manner as was the meat extract.

The bacteria in all cases were taken from fresh cultures, grown either upon agar-agar, gelatin or potato. The medium in which the bacteria grew was in all cases neutral.

The following are the tabulated results of a few of the gas-producing organisms of which a study was made :

TABLE NO. I.—BACILLUS AROMATICA.

	Temp.	Date.	Kind of sugar:	Gas present after days. 1	2	3	4	5	No. cc. taken.	Carbon dioxide. Per cent.	Hydrogen. Per cent.
1.	28°	July 23	Glucose.	0	87.5	173.	237.0		38.4	26.04	73.9
	18°	Oct. 1	Glucose.	0	...	...	...	45.4	15.7	26.68	73.3
2.	28°	Aug. 2	Cane....	0	39.5	...	93.8	122.9	18.7	23.5	76.4
	24°	Aug. 17	Cane....	0	...	...	...	....	32.2	21.8	78.0
3.		Aug. 10	Lactose.	0	0	0	0	0	0	0	0
		Aug. 15	Lactose.	0	0	0	0	0	0	0	0

TABLE NO. II.—MICROCOCCUS FROM CHEESE.

Date.	Kind of sugar.	Gas present after days. 1	2	3	4	5	No. cc. taken.	Carbon dioxide. Per cent.	Hydrogen. Per cent.
July 29	Glucose....	0	0	0	0	0	0	0	0
Sept. 22	Glucose....	0	0	0	0	0	0	0	0
Aug. 2	Cane.....	0	0	0	0	0	0	0	0
Sept. 22	Cane.....	0	0	0	0	0	0	0	0
Aug. 20	Lactose....	0	0	0	0	0	0	0	0
Sept. 15	Lactose....	0	0	0	0	0	0	0	0

TABLE NO. III.—JONES ANACROBE.

	Temp.	Date.	Kind of sugar.	Gas present after days. 1	2	3	4	5	No. cc. taken.	Carbon dioxide. Per cent.	Hydrogen. Per cent.
1.	28°	Sept. 6	Glucose..	76.6	134.6	....	...	..	24.3	27.8	72.1
		July 23	Glucose..	...	....	....	...	..	72.0	11.5	82.4
2.		Aug. 29	Cane	0	136.8	151.8	...	..	...	42.3	57.6
		July 8	Cane	...	....	....	...	..	85.0	44.7	55.3
3.		Aug. 6	Lactose..	0	0	0	0	.	0	0	0
		Sept. 15	Lactose..	0	0	0	0	..	0	0	0

TABLE NO. IV.—BACILLUS COLI COMMUNIS.

	Temp.	Date.	Kind of sugar.	Gas present after days. 1	2	3	4	5	No. cc. taken.	Carbon dioxide. Per cent.	Hydrogen. Per. cent.
1.	28°	Sept. 6	Glucose..	119.6	162.0	....	...	0	31.0	25.16	74.8
	22°	Sept. 30	Glucose..	....	76.6	....	...	...	45.0	23.2	76.8
2.	24°	Aug. 17	Cane	0	7.5	34.0	40.1	0	37.0	34.3	65.4
	22°	Oct. 15	Cane	0	....	....	5.2	...	28.0	31.6	68.4
3.	26°	Aug. 27	Lactose..	0	....	....	76.6	233.5	27.0	28.8	71.1
		Oct. 19	Lactose..	0	....	140.1	...	...	35.3	27.2	72.8

TABLE NO. V.—BACILLUS COLI COMMUNIS.[1]

Kind of sugar.	Per cent. of gas present after days. 1	2	3	4	5	6	7	Total gas at 20°-25°.	Reaction of bulb.	Carbon dioxide. Per cent.	Hydrogen. Per cent.
Glucose	28.0	44.0	47.0	..	..	..	..	9 days. 44.	.....	32.0	68.0
Cane	..	31.0	..	47.0	..	50.0	..	11 days. 43.	Acid.	36.5	63.5
Cane	..	7.0	..	13.0	..	15.0	16.0	10 days. 19.	Alkali.	23.0	77.0
Lactose.....	28.0	42.0	45.0	48.0	..	52.0	..	45.0	.....	37.0	35.0

[1] This table taken from Dr. Theobald Smith's work.

TABLE No VI. BACILLUS MESENTERICUS VULGATUS.

Temp.	Date.	Kind of sugar.	Gas present after days. 1	2	3	4	5	6	No. cc. taken.	Carbon dioxide. Per cent.	Hydrogen. Per cent.
22° C.	Oct. 2	Glucose..	0	0	0	0	0	0	0	0	0
24° C.	Oct. 7	Glucose..	0	0	0	0	0	0	0	0	0
25° C.	Sept. 15	Cane....	0	76.6	99.6	166.90	...	0	32.2	61.1	38.8
22° C.	Oct. 15	Cane....	.	...	76.6	.....	...	.	32.0	63.7	36.3
	Sept. 22	Lactose.	0	...	...	79.1	96.	.	22.8	23.5	76.5

A bacillus from butter-milk, not yet named, was used to inoculate a glucose peptone medium. The results being negative so far as gas development goes they are not tabulated. Action began in the separatory funnel, in course of twenty-four hours, and gas was quite rapidly developed. No gas was found in the flask after standing for four days and until action had ceased in the funnel. An examination of the medium in the flask showed that it was very markedly acid, showing that decomposition had taken place. The result is of value only as it shows that this bacillus can develop in the same medium with or without air and that the products vary in kind and amount.

STATEMENTS AND OBSERVATIONS ON THE TABULATED RESULTS.

1. As before mentioned, the media were neutral in all cases and were peptone bouillon, to which were added the different sugars, as noted in the tables.

2. The gas should not be analyzed until the action is about complete, owing to the fact of absorption of the carbon dioxide by the media. The first portions of the escaping gas were in the cases examined nearly pure hydrogon. Therefore in considering the total gaseous products the absorbed carbon dioxide should be taken into account. It was found that each cc. of the media absorbed on the average at ordinary temperatures eight cc. of carbon dioxide.

3. The temperature of the media has a marked effect, as is well known, on the rapidity and the time necessary for the development of the gas.

A difference of ten degrees made a difference of four days in the time of completing the action in the case of bacillus aromaticus.

4. It will be noticed that at the same temperature the action starts at different periods and that the maximum action occurs at different lengths of time from the time of inoculation.

5. Alcohol was produced by several of the bacteria studied. Bacillus aromaticus produced no alcohol, but did form lactic acid. The micrococcus from cheese produced no gas in any case, *i. e.*, not enough to more than saturate the media, although there was evidence of quite active growth.

6. The solutions were acid in all cases at the end of the action. Lactic acid was found in most cases.

7. Jones' anaerobe produced acetic and lactic acids with glucose, lactic acid, and only a trace of acetic acid with cane sugar. The alcohol was more abundant in the latter case.

8. Bacillus coli communis produced no alcohol, but did form lactic acid.

9. The bacillus mesentericus vulgatus rendered the media but slightly acid. No alcohol was produced.

10. It should be noted that the amount of carbon dioxide produced by the different micro-organisms studied varies with the kind of sugar used. See Table VI in reference to glucose and lactose. This evidently shows a smaller capacity to procure oxygen in one case than in the other.

11. Looking at the tabulated results under bacillus aromaticus, it will be seen that the organism produces gas in solutions of glucose and cane sugar, but it does not produce any gas in solutions of lactose sugar.

12. In comparing the total amounts of gases produced by different species of bacteria, it is seen that they are the most active in glucose media, with the exception of bacillus mesentericus vulgatus, which produces no gas in glucose media. This fact will serve to distinguish this bacillus from a larger number of others.

13. Bacillus aromaticus and Jones' anaerobe produce gas in solutions of glucose and cane sugar, but none in lactose sugar. Coli communis on the other hand produces gas in solutions of all three sugars, the largest amount of gas being produced in the lactose solution.

Where no record of gas production is made in the tables it

means that action practically ceased before this time was reached.

SOME OF THE INVESTIGATIONS, PRINCIPALLY OF GASEOUS PRODUCTS, OF THE GROWTH OF MICRO-ORGANISMS ARE SUMMARIZED HERE.

The following interesting conclusions from a study of stomach dilatation are drawn by Hoppe Seyler:

First. In not a few cases (thirteen out of twenty-two) there were present carbon dioxide and hydrogen.

Second. The formation of hydrogen depends on butyric ferment.

Third. The formation of this hydrogen goes on even when the fluid contents of the stomach reaches two per cent. of sodium chloride.

Fourth. By removal of sodium chloride there is usually a larger per cent. of carbon dioxide.

Fifth. By the yeast ferment carbon dioxide only is formed.

Sixth. Often the dilated stomach only contains the gases that have been swallowed.

Another very important investigation of the products of bacterial decomposition was made by Brieger, using the pneumococcus of Friedländer.

By growing this specific germ in suitable solution of grape or cane sugar, he obtained principally acetic, together with some formic and succinic acids and ethyl alcohol. The same products were also obtained by the growth of this organism in solution of calcium lactate and creatine. In bacillus ethaceticus the amount of alcohol and acetic acid stand to each other in virtually the same proportions as does that produced by bacillus pneumococcus, yet the absolute amounts produced are much less than in that of the latter.

There is often some difference in the day when fermentation begins, but Frankland, Stanley and Frew remark that (although there is some difference in the several series of experiments as to the precise period which elapses between the time of inoculation and the commencement of fermentation) the balance of evidence points to the glucose being the least, and to the mannitol and cane sugar being the most fermentable. In the glucose fer-

mentation of pneumococcus the proportion of hydrogen to carbon dioxide by volume shows that the gases are given off in approximately the same number of molecules of each, but when mannitol was used ten molecules of hydrogen to twelve molecules of carbon dioxide were produced. This larger proportional evolution of hydrogen in the case of mannitol is what might have been anticipated from a consideration of the larger per cent. of hydrogen in mannitol.

Gartner also made some very interesting investigations. He studied mainly one bacterium, which he called a new gas-producing pathogenic bacillus. He inoculated different media containing varying quantities of sugar and peptone, also media showing different reactions. Different products and different proportions of products were obtained in most cases. He states that a three per cent. glucose medium gives a relatively larger per cent. of gas than a one and one-half per cent. solution; also that an acid reaction hinders the production of gas and the total amount of gas is not as much as was formed from the same solution when it was neutral.

Another investigator of the gases produced by bacteria deserves mention, namely, Dr. Theobald Smith, of Washington.

Dr. Smith, in his analysis, used a fermentation tube devised by himself. The media and fermentation tubes were completely sterilized, after which the media was inoculated with specific germs. The gas was afterwards determined by using potassium hydroxide as an absorbing material for the carbon dioxide. The remainder of the gas he calls an explosive gas and assumes it to be hydrogen.

The types of bacteria which he took were bacillus coli communis, hog cholera bacillus, B. lactis aerogenes, bacillus of Friedlander, B. aedematis maligni, proteus vulgaris, B. cloacea and saccharomyces.

The result of his study of these micro-organisms was to show that the media conditions under which development took place modified the proportion of the gaseous products and the rapidity of their formation. His results also show that these facts may be used to a marked extent to determine species.

The following is a short bibliography of this subject.

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CHEMICAL VS. BACTERIOLOGICAL EXAMINATION OF POTABLE WATER.[1]

BY W. P. MASON.

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APROPOS of the recent articles upon this question, which have appeared in the English papers, it is noteworthy that there is a growing tendency among physicians and civil engineers to belittle the chemists opinion regarding the potability of a water, and to pin their faith exclusively upon what the bacteriologist may have to say upon the subject. This feeling is strengthened by the publication of the results of such trials as that undertaken by the London Local Government Board, in which it will be remembered, water samples purposely inoculated with typhoid germs, were sent for analysis to one of England's leading chemists and were by him pronounced pure.

Those who set special value upon such a "test" of methods as the above, and who consider it quite final as showing the

[1] Read before the New York Section.